Université Montpellier II, Sciences et Techniques du Languedoc - DRED - 3ème Cycle - HDR
Place Eugène Bataillon - 34095 Montpellier Cedex 5 - Tél. 04.67.14.30.23 –
Fax. 04.67.14.48.48

HABILITATION A DIRIGER DES RECHERCHES

NOM et Prénom : MAURICE Sandrine
Section CNU : 67
Titre des Travaux : Quelques polymorphismes sexuels

Date, heure et lieu de soutenance : vendredi 27 janvier 2012, 10h15, Université Montpellier 2 – SC 12.01

Résumé des ouvrages ou des travaux :
Dans le cadre général de l'évolution et du maintien des polymorphismes, mes travaux ont porté dans leur grande majorité sur les systèmes de reproduction. J'ai plus particulièrement étudié de façon théorique les conditions d'évolution de différents polymorphismes sexuels selon que le phénotype sexuel des individus est déterminé par des gènes nucléaire ou conjointement par des gènes nucléaires et cytoplasmiques. Mes travaux expérimentaux portent sur les plantes (angiospermes). Ils concernent la description et l'analyse de systèmes polymorphes et l'étude de l'allocation de ressources aux fonctions mâle et femelle. La biologie de la reproduction dans le cadre d'espèces invasives ou au contraire rares a aussi été abordée. Des études phylogénétiques et phylogéographiques ont été réalisées sur certaines des espèces étudiées.

Composition du Jury:

Nom et Prénom (en entier)	Grade	Etablissement
Mme SHYKOFF Jacqui	DR	CNRS - Université Paris-Sud
Mr TOUZET Pascal	Pr	Université Lille 1
Mme VIARD Frédérique	DR	CNRS - Station Biologique de Roscoff
MR VEKEMANS Xavier	Pr	Université Lille 1
Mr DAVID Patrice	DR	CNRS - Centre d'Ecologie Fonctionnelle et Evolutive
Mr THOMPSON John	DR	CNRS - Centre d'Ecologie Fonctionnelle et Evolutive

UNIVERSITE MONTPELLIER II

Habilitation à diriger les recherches

Dossier de candidature

Sandrine Maurice

Institut des Sciences de l'Evolution –UMR 5554

Université Montpellier II, place Eugène bataillon

34095 Montpellier

.

SOMMAIRE

CURRICULUM VITAE

ACTIVITES D'ENCADREMENT

PRODUCTION SCIENTIFIQUE

Sandrine MAURICE

Maître de Conférences à l'Université Montpellier II
Biologie Evolutive, section CNU 67

Principaux enseignements : Génétique des populations – Modélisation en biologie
Thèmes de recherche : Evolution des systèmes de reproduction – Biologie des invasions

née le 12 février 1966
nationalité française

Laboratoire Génétique et Environnement
Institut des Sciences de l'Evolution (ISEM)
Université Montpellier II, CC O65
34 095 MONTPELLIER Cedex 05

Tél.: 04 67 14 49 10
Fax: 04 67 14 20 32
smaurice@univ-montp2.fr

DIPLOMES-FORMATION-EMPLOIS

1994-2011	Maître de Conférences à l'Université Montpellier II (UM2).
1997-99	Maître de Conférences à l'Université de la Réunion (Mise à disposition).
1994	Attaché Temporaire d'Enseignement et de Recherche à l'Université de la Réunion.
1993	Stage post-doctoral au laboratoire Ecology and Evolution, University of Arizona, Tucson, USA (Bourse Lavoisier).
1992	Attaché Temporaire d'Enseignement et de Recherche à l'Université Paris-Sud Orsay.
1989-92	Thèse au laboratoire d'Evolution et Systématique des Végétaux, URA 1492, Université Paris-Sud Orsay (Bourse MRT et monitorat). Titre : "Evolution de la dioecie : aspects théoriques et aspects expérimentaux sur le genre *Silene*". Soutenue en septembre 92 à Montpellier. Directeur de thèse : P.-H. Gouyon.
1988	DEA "Sciences de l'Evolution et Ecologie" à l'USTL-Montpellier II. Diplôme d'Ingénieur Agronome de l'Institut National Agronomique Paris-Grignon.

RESPONSABILITES ADMINISTRATIVES et PEDAGOGIQUES

2001-08	Membre de la commission de spécialistes de l'Université Montpellier II, sections 24/67/68.
2004-08	Membre de la commission de spécialistes de l'Université de Bourgogne, sections 35/36/67.
2003-04	Responsable de la Maîtrise Biologie des Populations et Ecosystèmes de l'UM2.
2004-11	Responsable du parcours M1 Ecologie, Biodiversité et Evolution du Master Biologie, Géosciences, Agroressources et Environnement (BGAE) de l'UM2.
2004-11	Responsable de la spécialité Ecologie, Biodiversité et Evolution du Master Biologie (LMD1), puis co-responsable de la spécialité Biologie, Ecologie, Evolution, Environnement (LMD2) du master BGAE.
2004-11	Membre du bureau du Master BGAE.

ACTIVITES diverses liées à la RECHERCHE

Revues d'articles pour : AoB PLANTS, Acta oecologica, Journal of Evolutionary Biology, Proceedings of the Royal Society-Biological Sciences, Oikos, Heredity, American Naturalist, Australian Journal of Botany, Evolutionary Ecology.

Participation aux projets:

« Mécanismes d'adaptation des populations végétales à la fragmentation de l'habitat » – Actions Concertées Coordonnées – Sciences du Vivant (1996-1997, coordinateur du projet John THOMPSON, CEFE-Montpellier)

« Rubus alceifolius » – Financement CIRAD-Région Réunion (1999-2000, coordinateur Thomas LEBOURGEOIS, CIRAD-Montpellier)

« Mécanismes et conséquences de l'invasion de *Senecio inaequidens* en région méditerranéenne » – MATE (2001-2004, coordinateur du projet Jacques MAILLET, Supagro-Montpellier) – Responsable UM2 pour ce projet et obtention d'une bourse de thèse CNRS-Région sur le même thème.

« Evolution de la plasticité en environnement variable : le cas des systèmes de reproduction » – Conseil Scientifique UM2 (2011, responsable du projet).

Participation à l'organisation du Petit Pois Déridé 2002 (réunion du groupe de biologie et génétique des populations) à Montpellier : gestion des inscriptions.

ENCADREMENT d'ETUDIANTS (à partir de DEA/M2)

Encadrante principale

2000-03 Lucile LAFUMA – Thèse soutenue en décembre 2003
Titre : L'invasion de *Senecio inaequidens* (Asteraceae) en Europe: une approche évolutive.
Directeur de thèse : Isabelle Olivieri (ISEM - Montpellier)

2000 Lucile LAFUMA – DEA (Ecologie et Evolution, Montpellier) - septembre 2000
Titre : Déterminisme génétique du sexe et étude de l'allocation des ressources à la reproduction d'une espèce gyno-mono-dioïque, *Silene italica*.

1992 Christine DESFEUX – DEA (Ecologie, Orsay) - septembre 1992
Titre : La gynomonoecie chez *Silene nutans*.

Co-encadrante

2010-12 Bojana STOJANOVA – Thèse en cours
Sujet : Plasticité et système de reproduction chez une espèce cléïstogame, le lamium amplexicaule.
Co-encadrement : Pierre-Olivier Cheptou (CEFE - Montpellier)

2008 Edouard MUTSCHLER – Master 2 (Génétique-Evolution-Ecologie, Lille) – juin 2008
Titre : Origines et invasions des espèces du complexe de *Senecio inaequidens* (Asteraceae).
Co-encadrement : Laurent Amsellem (GEPV - Lille)

2001-04 Adeline CESARO – Thèse soutenue en juillet 2004
Titre : Le polymorphisme pour la longueur du style en tant qu'intermédiaire dans l'évolution de la distylie: Etudes expérimentales et théoriques chez *Narcissus assoanus* (Amaryllidacées).
Directeur de thèse : John Thompson (CEFE - Montpellier)

Participation à l'encadrement

2007 Loïc DORVILLE – Master 2 (Génétique-Evolution-Ecologie, Lille) – juin 2007

Titre : Localités d'origine et points d'introduction d'une plante invasive en Europe occidentale : *Senecio inaequidens* (Asteraceae).
Co-encadrement : Laurent Amsellem (GEPV - Lille)

2004 Isabelle LITRICO – Thèse soutenue en décembre 2004.
Titre : Evolution du genre sexuel et de la diversité génétique dans une succession primaire : l'étude d'*Antirhea borbonica* (Rubiaceae) sur les coulées de lave à la Réunion.
Directeur de thèse : John Thompson (CEFE - Montpellier)

1999-2002 Stéphane BARET – Thèse soutenue en décembre 2002.
Titre : Mécanismes d'invasion de *Rubus alceifolius* à l'île de la Réunion : interaction entre facteurs écologiques et perturbations naturelles et anthropiques dans la dynamique d'invasion.
Directeur de thèse : Jacques Figier (Laboratoire de Biologie Végétale de l'Université de la Réunion)

1997-99 Thierry PAILLER – Thèse soutenue en décembre 1997.
Titre : L'hétérostylie dans l'archipel des Mascareignes : Présence, maintien et évolution.
Directeur de thèse : John Thompson (CEFE - Montpellier)

1992-96 Christine DESFEUX – Thèse soutenue en mai 1996.
Titre : Evolution du système de reproduction dans le genre *Silene*.
Directeur de thèse : Pierre-Henri Gouyon (ESE - Orsay)

PARTICIPATION à des JURYS de THESE

GARRAUD Claire. Evolution de la gynodioécie-gynomonoecie – Approches expérimentales chez *Silene nutans* et approche théorique. Soutenue le 11 mars 2011 à l'Université Paris Sud XI.

MONTY Arnaud. Sources de variation phénotypiques des traits d'histoire de vie d'une espèce invasive, *Senecio inaequidens* DC. (Asteraceae). Soutenue le 22 avril 2009 à l'Université des Sciences Agronomie de Gembloux, Belgique.

CAÑO Lidia. Factores ecologicos y evolutivos que regulan la capacidad invasora de *Senecio pterophorus* D.C. y *S. inaequidens* D.C. (Asteraceae) en el NE de la peninsula ibérica. Soutenue le 15 juillet 2007 à l'Université Autonome de Barcelone.

GARCIA I SERRANO Hector. Comparative studies of alien and native Senecio species differing in invasiveness and distribution range. Soutenue le 14 octobre 2004 à l'Université Autonome de Barcelone.

CESARO Adeline. Le polymorphisme pour la longueur du style en tant qu'intermédiaire dans l'évolution de la distylie: Etudes expérimentales et théoriques chez *Narcissus assoanus* (Amaryllidacées). Soutenue le 9 juillet 2004 à l'Université Montpellier II.

LAFUMA Lucile. L'invasion de *Senecio inaequidens* (Asteraceae) en Europe: une approche évolutive. Soutenue le 12 décembre 2003 à l'Ecole Nationale Supérieure d'Agronomie de Montpellier.

COMMUNICATIONS

TONNABEL, J., T. VAN DOOREN, A. MIGNOT, S. MAURICE, O. RONCE, A. REBELLO, J. MIDGLEY, P. HACCOU & I. OLIVIERI. Modélisation de l'allocation optimale des ressources à la sérotinie et analyses de viabilité des populations sous différents régimes de feu. Ecologie 2010, Montpellier (F-34), septembre 2010.

MAURICE, S &. L. LAFUMA. Self-incompatibility in the *Senecio inaequidens* - *S. madagascariensis*

complex in its native and invaded ranges. Congress of the European Society for Evolutionary Biology, Turin (I), août 2009.

MAURICE, S & J. MAILLET. Mécanismes et conséquences de l'invasion de *Senecio inaequidens* en région méditerranéenne. Colloque de restitution du programme Invasions Biologiques, Molliets (F-33), octobre 2006.

MAURICE, S., M. ALLEAUME-BENHARIRA, L. LAFUMA, O. RONCE & I. OLIVIERI. Comparative population genetics of native and invasive populations of *Senecio inaequidens*: models and data. Invasive Plant in Natural and Managed Systems: Linking science and management, Fort Lauderdale (USA), novembre 2003.

LAFUMA, L., O. RONCE, I. BOURRIE, F. SHAW, M. ALLEAUME-BENHARIRA & S. MAURICE. (Poster) Evolution of genetic diversity during the invasion of the European weed Senecio inaequidens (Asteraceae). Congress of the European Society for Evolutionary Biology, Leeds (UK), août 2003.

LAFUMA, L., L. VIMOND & S. MAURICE. Identity and origin of the polyploïd invasive weed *Senecio inaequidens* (Asteraceae) in Europe. ESF workshop. "Biological invasions in terrestrial ecosystems : an evolutionary perspective", Leipzig (G), 2002.

MAURICE, S. Sex Expression and Stability in Silene italica. Evolutionary biology workshop "Breeding systems: evolutionary issues and novel approaches", La Fouly (CH), 2002.

MAURICE, S. The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphism. Fifth congress of the European Society for Evolutionary Biology, Edinburgh (UK), septembre 1995.

MAURICE, S. & T. H. FLEMING. (poster) What's going on in the trioecious cactus *Pachycereus pringlei*? Estimates of relative fertilities of males, females and hermaphrodites. Réunion annuelle des sociétés "American Society of Naturalists", "Society of Systematic Biologists", "Society for the Study of Evolution", Snowbird (USA), juin 1993.

DESFEUX, C., S. MAURICE & J.-P. HENRY. Que font les plantes gynomonoïques de *Silene nutans* de leurs économies? Groupe de Génétique et Biologie des Populations (GGBP) à Gif (F-91), septembre 1993.

MAURICE, S. From hermaphrodism to dioecy. Conférences Jacques Monod à Aussois (F-73), avril 1992.

MANICACCI, D. & S. MAURICE. Allocation sexuelle des hermaphrodites en gynodioecie : modélisation et données en populations naturelles. GGBP à Lille (F-59), septembre 1991.

HENRY, J.-P., C. DESFEUX & S. MAURICE. Etude d'une espèce gynomonoïque *Silene nutans*: les femelles sont-elles des monstres? GGBP à Lille (F-59), septembre 1991.

MAURICE, S., P.-H. GOUYON & HERMAPHROPOULOS. Les interactions nucléo-cytoplasmiques et la dioecie. GGBP à Bordeaux (F-33), septembre 1990.

MANICACCI, D., M.-C. ANSTETT, S. MAURICE, A. ATLAN, P.-H. GOUYON & D. COUVET. Dynamique oscillatoire de la stérilité-mâle : des données à la modélisation. GGBP à Bordeaux (F-33), septembre 1990.

DESPRES, L., S. MAURICE & C. COMBES. Sexe et schistosomes. GGBP à Bordeaux (F-33), septembre 1990.

MAURICE, S. & F. KJELLBERG. Les figuiers en conditions de saisonnalité. GGBP à Paimpont (F-35), septembre 1988.

ARTICLES PUBLIES ou ACCEPTES dans des revues indexées

Le nom des étudiants en thèse au moment des travaux est sous-ligné.

A23 - MONTY, A., S. MAURICE & G. MAHY. 2010. Phenotypic traits variation among native diploid, native tetraploid and invasive tetraploid Senecio inaequidens DC. (Asteraceae). ***Biotechnologie, Agronomie, Société et Environnement*** 14: 627-632.

A22 - NOËL, F., S. MAURICE, A. MIGNOT, S. GLEMIN, D. CARBONNEL, F. JUSTY, I. GUYOT, I. OLIVIERI & C. PETIT. 2010. Interaction of climate, demography and genetics: a ten-year study of *Brassica insularis*, a narrow endemic mediterranean species. ***Conservation Genetics*** 11: 509-526.

A21 - GLEMIN, S., C. PETIT, S. MAURICE & A. MIGNOT. 2008. Consequences of low mate availability in the rare self-incompatible species *Brassica insularis* (Brassicaceae). ***Conservation Biology*** 22: 216-221.

A20 - DUFAŸ, M., P. TOUZET, S. MAURICE & J. CUGEN. 2007. Modelling the maintenance of a male-fertile cytoplasm in a gynodioecious population? ***Heredity*** 99: 349-356.

A19 - LAFUMA, L. & S. MAURICE. 2007. Increase in mate availability without loss of self-incompatibility in the invasive species *Senecio inaequidens* (Asteraceae). ***Oikos*** 116: 201-208.

A18 - LAFUMA, L. & S. MAURICE. 2006. Reproductive characters in a gynodioecious species, *Silene italica* (Caryophyllaceae), with attention to the gynomonoecious phenotype. ***Biol. J. Linn. Soc.*** 87: 583-591.

A17 - EHLERS, B., S. MAURICE & T. BATAILLON. 2005. Sex inheritance in gynodioecious species: a polygenic view. ***Proc. R. Soc. Lond. B*** 272: 1795-1802.

A16 - CESARO, A.C., S.C.H. BARRET, S. MAURICE, B.E. VAISSIERE & J.D. THOMPSON. 2004. An experimental evaluation of self-interference in *Narcissus assoanus*: functional and evolutionary implications. ***J. Evol. Biol.*** 17: 1367-1376.

A15 - BARET, S., S. MAURICE, T. LE BOURGEOIS & D. STRASBERG. 2004. Altitudinal variation in fertility and vegetative growth in the invasive plant *Rubus alceifolius* Poiret (Rosaceae) on Reunion Island. ***Plant Ecology*** 172: 265-273.

A14 - LAFUMA, L., K. BALKWILL, E. IMBERT, R. VERLAQUE & S. MAURICE. 2003. Ploidy level and origin of the European invasive weed *Senecio inaequidens* (Asteraceae). ***Plant Syst. Evol.*** 243: 59-72.

A13 - PAILLER, T., S. MAURICE & J.D. THOMPSON. 2002. Pollen transfer patterns in a distylous plant with overlapping pollen-size distributions. ***Oikos*** 99: 308-316.

A12 - MAURICE, S. 1999. Gynomonoecy in *Silene italica* (Caryophyllaceae): sexual phenotypes in natural populations. ***Plant biol.*** 1: 346-350.

A11 - MAURICE, S., C. DESFEUX, A. MIGNOT & J.-P. HENRY. 1998. Reproductive biology of two subspecies of *Silene acaulis* (Caryophyllaceae). ***Can. J. Bot.*** 76: 478-485.

A10 - FLEMING, T. H., S. MAURICE & J. L. HAMRICK. 1998. Geographic variation in the breeding system and the evolutionary stability of trioecy in *Pachycereus pringlei* (Cactaceae). ***Evolutionary Ecology*** 12: 279-289.

A9 - DESFEUX, C., S. MAURICE, J.-P. HENRY, B. LEJEUNE & P.-H. GOUYON. 1996. The evolution of reproductive systems in the genus *Silene*. ***Proc. R. Soc. Lond. B*** 263: 409-414.

A8 - MAURICE, S. & T. H. FLEMING. 1995. The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphism. ***Oikos*** 74: 55-60.

A7 - DESPRES, L. & S. MAURICE. 1995. The evolution of dimorphism and separates sexes in Schistosomes. ***Proc. R. Soc. Lond. B*** 262: 175-180.

A6 - MAURICE, S., E. BELHASSEN, D. COUVET & P.-H. GOUYON. 1994. Evolution of dioecy: can nuclear-cytoplasmic interactions select for maleness? ***Heredity*** 73: 346-354.

A5 - FLEMING, T. H., S. MAURICE, S. L. BUCHMANN & M. D. TUTTLE. 1994. Reproductive biology and the relative male and female fitness in a trioecious cactus, *Pachycereus pringlei* (Cactaceae). ***Am. J. Bot.*** 81: 858-867.

A4 - MAURICE, S., D. CHARLESWORTH, C. DESFEUX, D. COUVET & P.-H. GOUYON. 1993. The evolution of gender in hermaphrodites of gynodioecious populations with nuclear-cytoplasmic male-sterility. ***Proc. R. Soc. Lond. B*** 251: 253-261.

A3 - GARNIER, P., S. MAURICE & I. OLIVIERI. 1993. Costly pollen in maize. ***Evolution*** 47: 946-949.

A2 - MAURICE, S. 1992. Maintenance of nucleo-cytoplasmic polymorphism under dioecious reproductive system. ***J. Theor. Biol.*** 154: 239-247.

A1 - KJELLBERG, F. & S. MAURICE. 1989. Seasonality in the reproductive phenology of *Ficus*: its evolution and consequences. ***Experientia*** 45: 653-660.

AUTRES PUBLICATIONS : Chapitres d'ouvrages – Articles de vulgarisation – Articles dans des revues non indexée

LENORMAND, T., D. ROZE, P.-O. CHEPTOU & S. MAURICE. 2010. L'évolution du sexe : un carrefour pour la biologie évolutive. In : F. Thomas, T. Lefevre, M. Raymond, eds. ***Biologie Evolutive***. De Boeck. pp 293-335.

MAURICE, S. Le séneçon du Cap en région Languedoc-Roussillon. ***Les dossiers d'AGROPOLIS INTERNATIONAL*** – Lutte Biologique, biodiversité et écologie en protection des plantes. Mars 2007.

GOUYON, P.-H., S. MAURICE, X. REBOUD & I. TILL-BOTTRAUD. Le sexe, pour quoi faire? ***La Recherche***, janvier 1993.

GOUYON, P.-H., S. MAURICE, X. REBOUD & I. TILL-BOTTRAUD. L'évolution du sexe. ***Aspects de la Recherche*** *1990. Université de Paris-Sud.*

ACTIVITES D'ENSEIGNEMENT

ACTIVITES D'ENSEIGNEMENT

Lorsque je ne précise rien, c'est que j'ai créé seule les enseignements donnés, sinon je mentionne qu'ils étaient existants ou avec qui je les ai créés.

1989-92 Université Paris-Sud Orsay Monitrice (2 ans) puis ATER (6 mois)

Biologie végétale
Quelques TP (existants) en DEUG

Génétique des populations
TD en Licence de génétique, quelques TD pour le DEA "Sciences de l'Evolution et Ecologie" de l'Université de Montpellier II ainsi que pour le module "Mécanismes en Evolution" de Maîtrise d'Orsay

Génétique formelle
TP-TD (existants) en Licence de génétique

J'ai eu la chance de faire un monitorat que je considère comme idéal : pour partie encadré et pour partie assez libre. En génétique formelle, j'assistais à une séance (T.P. et T.D. existants sur les sujets suivants : étude de mutants déficients respiratoires et cartographie de mutations mitochondriales chez la levure, isolement et caractérisation de mutations létales chez la drosophile) et je faisais la séance suivante avec l'enseignante présente dans ma salle. Pour la génétique des populations, du fait du changement de professeur de génétique des populations, il a fallu en grande partie repenser les T.D. avec l'enseignante titulaire (Marie-Thérèse PEIGNE) et d'autres étudiants. Nous avons donc conçu des T.D. sur les thèmes suivants : probabilités et statistiques, bases de la génétique des populations (loi de Hardy-Weinberg, sélection, dérive, mutation, régime de reproduction). Des séances sur ordinateur pour la compréhension de la dérive ont également été mises en place.

1994, 1997-99 Université de la Réunion ATER (8 mois) puis MC détachée

Mathématiques et algorythmique
TD (existants) en DEUG SSM(A) et SNV(B)

Statistiques
TD (existants) en DEUG SNV

Biologie évolutive
Cours et TD en Licence et Maîtrise de Biologie

1994-2011 Université Montpellier 2 MC

Principales activités actuelles :

Génétique des populations
TD en M1 **E**cologie, **B**iodiversité et **E**volution

Ecologie théorique
Cours et TD en M1 EBE (cours créés avec l'aide d'O. RONCE (CNRS) ; TD en majorité créés par J.-B. FERDY et F. MUNOZ)

Modélisation en biologie
Cours et TP en M2 **B**iologie de l'**E**volution et **E**cologie (responsable du module)

Encadrement des stages d'initiation à la recherche
en M1 EBE (*responsable du module*)

Par le passé :

Génétique des populations, génétique évolutive

Cours et TD en Licence de Physiologie végétale et Licence de Physiologie animale (*responsable du module*)

TD en Maîtrise **B**iologie des **P**opulations et **E**cosystèmes (création du polycopié d'exercices avec I. OLIVIERI)

Cours et TD en DEA **E**volution et **E**cologie, DEA **R**essources **P**hytosanitaires et **I**nteractions (avec J. RONFORT (INRA))

Cours en DEA **B**iologie, **E**volution et **C**ontrôle des **P**opulations –Tours

Modélisation en biologie

Cours, TD et encadrement de projet en Maîtrise BPE (avec C. ALIAUME)

Cours et TP en DEA BEE (*responsable du module*)

Biostatistiques

TD et TD en M1 EBE (existants - création J.-B. FERDY et F. CARCAILLET)

Morphologie Végétale

TP (existants) en DEUG

Phylogénie

Cours et TD en DEUG (existants - création E. DOUZERY)

Génétique formelle

TD en DEUG (création du polycopié d'exercices avec A. MIGNOT)

Biologie théorique et génétique

TD en DEUG - Ce module optionnel de DEUG a pour but de montrer aux étudiants ce que la formalisation mathématique peut apporter à l'étude et à la compréhension de phénomènes biologiques (création I. OLIVIERI). J'ai élaboré, pour ce module, des T.D. sur les modèles de base utilisés en dynamique des populations et un T.D. sur la dérive génétique

Ethologie

1996-98 Il s'agissait de donner aux étudiants de Maîtrise BPE une introduction à l'éthologie, notamment à l'éthologie expérimentale, avant le recrutement d'enseignants compétents dans cette discipline. Deux T.P. sur le comportement ont été effectués sur le phénomène d'habituation et sur le comportement de toilettage (création A. MIGNOT). Trois T.D. sur discussion d'articles et d'expérimentations ont été montés : sur la sélection sexuelle, sur les systèmes de reproduction, sur la socialité et la colonialité (avec A. MIGNOT).

Projets tutorés de Licence

Il s'agit d'encadrer des trinômes d'étudiants de L3 dans leur recherche sur un thème proposé.

Je vais dire quelques mots sur le module « **Formalisation et modélisation de problèmes biologiques** » - module de 25 heures conjoint M2 BEE / Ecole Doctorale - car c'est mon enseignement le plus exigeant, en tout cas celui qui chaque année me donne à la fois le plus de sueurs froides et de satisfaction.

La modélisation est une technique devenue omniprésente en biologie, fondamentale ou appliquée, notamment dans les domaines qui concernent mes étudiants : l'écologie, l'évolution, la gestion des ressources génétiques. Mais elle reste pour certains mystérieuse voire rébarbative. Je note néanmoins depuis quelques années une nette amélioration, à la fois de la perception de la matière et du niveau des étudiants, due je pense au développement de l'enseignement des statistiques et à l'introduction des modèles dans l'enseignement d'écologie.

Le but est donc de dédramatiser la modélisation et de fournir des bases - pour ceux qui auront à employer l'outil ou tout simplement pour mieux comprendre les articles théoriques. Je donne un aperçu d'un certain nombre de concepts : comment formaliser un problème, l'approche déterministe, l'approche stochastique, le test de modèle ; et techniques : obtention de maxima (*utilisation des dérivés*), obtention d'équilibre (*résolution d'équations*), analyse de stabilité (*dérivées partielles*, *calcul matriciel*), équations différentielles et leurs résolutions analytiques ou numériques,

itération d'équations, utilisation des tirages aléatoires et simulations de Monte Carlo. Une partie du jeu étant de leur montrer qu'ils ont déjà les outils de base (*en italique précédemment*) au fond de leur tête et que oui, toutes ces années de mathématiques peuvent leur servir. Plutôt que de donner un descriptif exhaustif des techniques, je préfère aborder quelques situations relativement simples pour que l'étudiant puisse faire lui-même (avec un peu d'aide) la modélisation du processus et les calculs ou la programmation nécessaires à l'étude du comportement de son modèle. Les logiciels utilisés ont été successivement Turbo Pascal, Delphi, Mathematica et actuellement R. Les trois derniers ayant été appris pour ce module, on peut dire que l'enseignement maintient en forme aussi sûrement que la recherche !
Comme la correction des équations et programmes des étudiants est assez consommatrice de temps, j'effectue quand c'est possible cet enseignement secondée par un intervenant. Ces intervenants changent selon les ans et leurs apports successifs sont incorporés et ont grandement modelés et améliorés le module (T. BATAILLON, A. TSITRONE – chercheurs INRA, S. GLEMIN – MC, V. CALCAGNO – ATER).
J'ai effectué une variante de ce module pour les programmes doctoraux de la Faculté des Sciences de l'Université de Lisbonne en juillet 2010 (Advanced courses 2010 – Modelling in Evolution and Population Genetics – 30h).

ACTIVITES DE RECHERCHE

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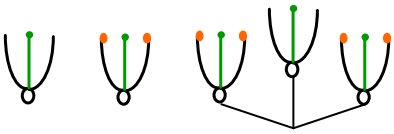
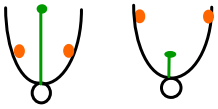
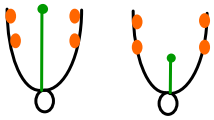
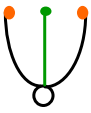
Tableau 1 : Terminologie des systèmes de reproduction polymorphes. La fonction femelle est représentée en vert, la fonction mâle en orange.

Terminologie	Composition des populations	
<u>Polymorphisme sur les fonctions mâle et femelle</u>		
2 types		
Dioecie	Femelles / Mâles	
Gynodioecie	Femelles / Hermaphrodites	
Androdioecie	Mâles / Hermaphrodites	
3 types ou 2 types dont un variable		
Trioecie	Femelles / Mâles / Hermaphrodites	
Subdioecie	Femelles / Mâles / Hermaphrodites en faible fréquence ou très semblables aux mâles (i.e. avec une faible production de fruit)	
Gynomono-dioecie	Femelles / Hermaphrodites (éventuellement) / Gynomonoïques (hermaphrodites avec des fleurs H et F)	
<u>Polymorphisme sur l'expression de l'hermaphroditisme</u>		
2 types		
Distylie	Hermaphrodites à style long et anthères basses / Hermaphrodites à style court et anthères hautes	
Polymorphisme de longueur de style	Hermaphrodites à style long / Hermaphrodites à style court (même niveau d'anthères pour tout le monde)	
3 types		
Tristylie	Hermaphrodites à style long et anthères basses et moyennes / Hermaphrodites à style moyen et anthères basses et hautes / Hermaphrodites à style court et anthères hautes et moyennes	
n types		
Système d'incompatibilité	Hermaphrodites morphologiquement semblables mais différant génétiquement par leurs allèles d'incompatibilité (S-allèles)	

ACTIVITES DE RECHERCHE

Dans le cadre général de l'évolution et du maintien des polymorphismes, mes travaux ont porté dans leur grande majorité sur les systèmes de reproduction. J'ai plus particulièrement étudié de façon théorique les conditions d'évolution de différents polymorphismes sexuels (voir tableau 1 ci-contre) selon que le phénotype sexuel des individus est déterminé par des gènes nucléaires (A1, A7, A8) ou conjointement par des gènes nucléaires et cytoplasmiques (A2, A4, A6, A17, A20). Mes travaux expérimentaux portent sur les plantes (angiospermes). Ils concernent la description et l'analyse de systèmes polymorphes (A5, A10, A11, A12, A13, A16) et l'étude de l'allocation de ressources aux fonctions mâle et femelle (A3, A18). La biologie de la reproduction dans le cadre d'espèces invasives ou au contraire rares a aussi été abordée (A15, A19, A21, A22, A23). Des études phylogénétiques (A9) et phylogéographiques (A14) ont été réalisées sur certaines des espèces étudiées.

Les mots « types » ou « types sexuels » employés dans la suite de ce texte désignent des phénotypes, et pas forcément des génotypes. De même, on appellera polymorphisme la coexistence de plusieurs phénotypes, on précisera donc lorsqu'il s'agira d'un polymorphisme génétique. Tous les raisonnements présentés seront par défaut valides pour un déterminisme du type sexuel nucléaire et sans contrainte particulière. Sinon le déterminisme envisagé sera précisé.

Les données à expliquer:

Un résumé de la terminologie simplifiée des systèmes de reproduction polymorphes est donné dans le tableau 1 ci-contre. J'ai distingué les polymorphismes portant de façon évidente sur l'allocation aux fonctions mâle et femelle des polymorphismes portant a priori sur des hermaphrodites exprimant les deux fonctions de façon équilibrée – c'est une simplification dans la mesure où rien ne garantit que les différents types d'hermaphrodites ont le même succès, voire la même allocation a priori, dans les fonctions mâle et femelle. Il existe d'autres polymorphismes sur l'expression de l'hermaphrodisme dont je ne parlerai pas ici comme l'énantiostylie (torsion du style vers la droite ou vers la gauche) et le polymorphisme de couleur de fleur.

Je m'intéresse actuellement plus particulièrement à la coexistence de plus de deux types sexuels, néanmoins j'ai d'abord travaillé sur les conditions de maintien et d'évolution des systèmes gynodioïque et dioïque. De plus une partie des mécanismes invoqués pour expliquer la coexistence de trois types est aussi impliquée dans la coexistence de deux types. Je ferai donc un rappel des conditions d'évolution des polymorphismes à deux types, avant de détailler du point de vue des données et du point de vue théorique les systèmes à trois phénotypes.

Systèmes de reproduction stables Encadré 1
lorsque le déterminisme du sexe est nucléaire et sans contrainte

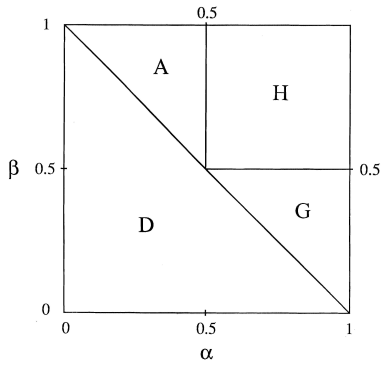
Formalisme en fertilités relatives fixes (Charlesworth & Charlesworth, 1978)

Soient AF=1+k, la production de graines des femelles par rapport à celle des hermaphrodites et
AM = 1+K, la production de pollen des mâles par rapport à celle des hermaphrodites.
Des femelles sont présentes dans les populations hermaphrodites si AF>2.
Des mâles sont présents dans les populations hermaphrodites si AM>2.
Des mâles remplaceront les hermaphrodites dans une population gynodioïque si K>1/k.

Pour faire plus facilement le lien avec le formalisme des fitness-set, on utilisera plutôt :
α : production de pollen des hermaphrodites relativement aux mâles (=1/AM) et
β : production de graines des hermaphrodites relativement aux femelles (=1/AF).

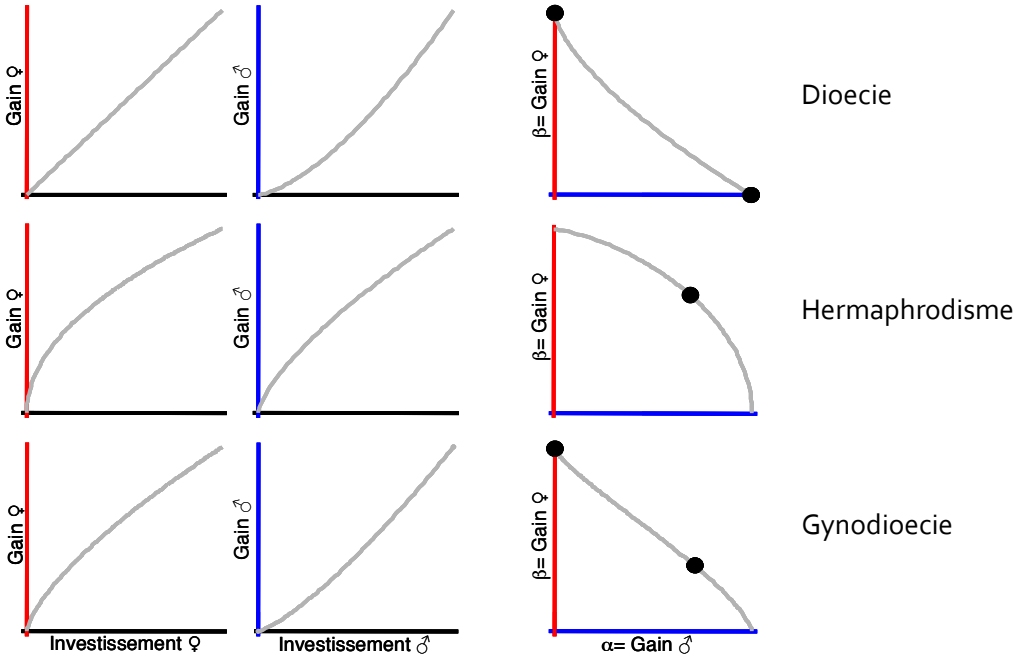
Les systèmes de reproduction stables sont alors représentés, en fonction de α et β, dans le graphe ci-contre, avec :

H : Hermaphrodisme,
D : Dioecie,
G : Gynodioecie,
A : Androdioecie.



Formalisme des fitness-sets (Charnov, 1982)

L'hypothèse de base est que la quantité totale de ressources allouée à la reproduction est limitée, et donc que plus un individu investit dans la fonction mâle, moins il investit dans la fonction femelle. Les deux premières colonnes ci-dessous représentent les gains (en nombre ou efficacité des gamètes) selon l'investissement dans les fonctions femelle et mâle (gain = investissement^k, k<1 donnant une fonction saturante). De ces courbes de gains découlent la forme du fitness-set qui représente les possibilités de réalisation des fonctions sexuelles pour toutes les stratégies d'investissement possibles (dernière colonne). La forme de celui-ci détermine les types sexuels présents.



1. Coexistence de deux phénotypes sexuels

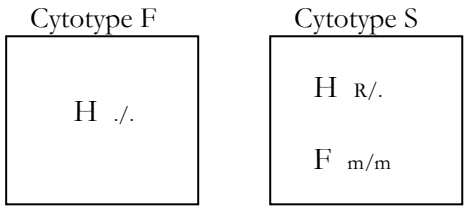
1.1. Coexistence de deux phénotypes sexuels correspondant à deux catégories génotypiques

La coexistence de mâles et de femelles (en absence d'hermaphrodites) ne pose pas de problème car le fait que les deux fonctions soient nécessaires à la reproduction crée une fréquence-dépendance qui maintient obligatoirement le polymorphisme. Lorsqu'on a des mâles et des hermaphrodites, ou bien des femelles et des hermaphrodites, c'est-à-dire qu'il y a une fonction en commun entre les deux types, la coexistence est moins évidente et il existe des conditions sur les fertilités des types pour la fonction commune pour que le polymorphisme soit maintenu. Ces conditions, qui sont donc celles de stabilité des différents systèmes de reproduction, ont été calculées essentiellement par Charnov (Charnov *et al.*, 1976 ; Charnov, 1982) et Charlesworth & Charlesworth (1978, entre autres). Leurs résultats, qui serviront de référence, sont résumés en encadré 1. Le premier utilise des modèles phénotypiques, c'est-à-dire qu'il ne suppose pas de déterminisme génétique particulier au type sexuel, tandis que les seconds utilisent parfois des modèles génétiques. Dans les deux cas, l'analyse se base sur le critère d'invasion, c'est-à-dire qu'on regarde dans quelles conditions une stratégie (ici un type sexuel ou une association de types sexuels) est stable face à une autre stratégie apparaissant en fréquence rare dans la population (voir plus loin l'encadré 3- modèles phénotypiques, pour une illustration de ces méthodes). On peut d'autre part distinguer les modèles dans lesquels l'allocation sexuelle des individus est fixe des modèles dans lesquels toutes les allocations sexuelles sont possibles (encadré 1). Ces différents types de modèles donnent des résultats cohérents mais parfois différents et je donnerai des illustrations de ces différences au cours de l'exposé de mes travaux.

Les résultats de l'encadré 1 sont issus de modèles phénotypiques et ne sont valables que lorsque le déterminisme du sexe est nucléaire. Or il est connu que le phénotype femelle est souvent induit par des gènes cytoplasmiques mitochondriaux (dits de stérilité-mâle) car ceux-ci n'étant transmis que par les ovules, ils sont sélectionnés positivement dès que les femelles sont meilleures que les hermaphrodites, même très légèrement, dans l'accomplissement de la fonction femelle. De nombreuses espèces gynodioïques possèdent de fait un déterminisme du sexe nucléo-cytoplasmique, c'est-à-dire faisant intervenir des gènes cytoplasmiques de stérilité-mâle et des gènes nucléaires restaurant cette fertilité (Couvet *et al.*, 1990 ; Budar *et al.*, 2003). Une abondante littérature théorique est disponible sur ce sujet (voir A20 pour une synthèse) et j'ai travaillé sur le maintien des femelles face aux hermaphrodites lorsque le déterminisme du sexe est asymétrique (un cytotype fertile et un cytotype stérile - voir encadré 2) ou quantitatif. Le déterminisme nucléo-cytoplasmique du sexe se prête mal aux études purement analytiques, notamment car un même phénotype global (par exemple hermaphrodite) se déclinera en plusieurs sous-phénotypes (hermaphrodites avec des fertilités mâle et/ou femelle différentes) et que de plus chaque sous-phénotype peut correspondre à plusieurs génotypes (encadré 2) ; à l'inverse l'effet d'un gène donné dépendra du reste du contexte génétique donc le critère d'invasion n'est pas toujours facile à mettre en oeuvre. Ces études sont donc souvent numériques : on itère des équations de récurrence sur les fréquences génotypiques permettant de passer d'une génération à l'autre jusqu'à obtention d'un équilibre, en vérifiant l'influence - ou l'absence d'influence - des fréquences génotypiques de départ sur l'équilibre obtenu (voir plus loin l'encadré 4- modèles génétiques, pour une illustration de ces méthodes).

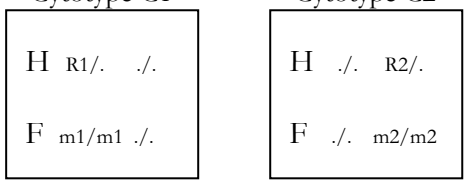
Exemples de déterminismes nucléo-cytoplasmiques du sexe Encadré 2

Déterminisme asymétrique F/S : un cytotype fertile, un cytotype stérile
Proposé chez *Beta maritima*



Tous les individus portant le cytotype F sont mâle-fertiles (H pour hermaphrodite). Les individus portant le gène cytoplasmique de stérilité S sont mâle-fertiles s'ils portent le gène de restauration (R, ici considéré dominant) sinon ils sont femelles (F).

Déterminisme symétrique C1/C2 : deux cytotypes stériles
Proposé chez *Plantago lanceolata*



Les individus sont mâle-fertiles s'ils portent le gène de restauration spécifique de leur gène cytoplasmique de stérilité. Des hermaphrodites et des femelles sont donc présents dans les deux compartiments cytoplasmiques.

Les paramètres intervenant dans les modèles sont 1- l'avantage femelle, c'est-à-dire l'augmentation de fertilité femelle liée au fait de ne faire que la fonction femelle (par réallocation des ressources non consommées par la fonction mâle, ou par évitement de la dépression de consanguinité dans les modèles qui considèrent l'autofécondation possible pour les hermaphrodites) ; 2- l'effet du cytotype au delà de son effet principal sur la production ou non de pollen : par exemple il est en général considéré que les gènes de stérilité-mâle perturbent le métabolisme mitochondrial et donc que pour un même phénotype sexuel, les individus portant un cytotype fertile ont des fertilités plus élevées que les individus portant un cytotype de stérilité ; 3- le coût pleïotropique de la restauration car les restaurateurs perturbent les interactions nucléo-cytoplasmiques.

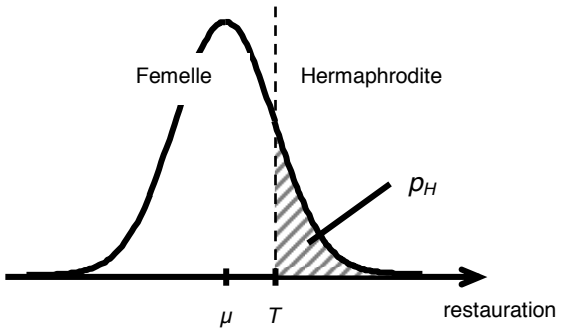
Exemple de fertilités des individus C1 selon leur génotype nucléaire et les paramètres du modèle (AF : avantage femelle, W : effet du cytotype, d : coût du restaurateur) :

		Coût constitutif		Coût exprimé		Coût silencieux	
Locus 1	Locus 2	Fertilité femelle	Fertilité mâle	Fertilité femelle	Fertilité mâle	Fertilité femelle	Fertilité mâle
R1/.	R2/.	W_1	$W_1(1-d_1)(1-d_2)$	W_1	$W_1(1-d_1)$	W_1	$W_1(1-d_2)$
	m2/m2	W_1	$W_1(1-d_1)$	W_1	$W_1(1-d_1)$	W_1	W_1
m1/m1	R2/.	W_1.AF	0	W_1.AF	0	W_1.AF	0
	m2/m2	W_1.AF	0	W_1.AF	0	W_1.AF	0

Dans les cas ci-dessus on a représenté le cas d'un gène de restauration dominant, aussi bien dans son effet sur la restauration de la production de pollen que dans son effet sur le coût de la restauration, et ce coût affectait seulement la fonction mâle des individus hermaphrodites portant ce gène. Les gènes de restauration sont le plus souvent dominants pour leur effet sur le type sexuel mais des gènes récessifs ont aussi été décrits. Quand au coût de ces gènes, il est très difficile à mettre en évidence et plusieurs modalités ont été proposées : coût sur la fonction mâle seulement car ils interviennent au niveau de la production de pollen, sur la fonction femelle ou sur les deux, coût présent dans tous les contextes cytoplasmiques (dit coût constitutif), coût s'exprimant seulement en présence du cytotype de stérilité associé (dit coût exprimé), coût s'exprimant seulement dans les autres cytotypes (dit coût silencieux).

Déterminisme quantitatif

L'idée d'un déterminisme quantitatif du sexe vient notamment de données sur le thym ayant montré que les femelles les plus proches morphologiquement des hermaphrodites donnaient un pourcentage plus élevé d'hermaphrodites dans leur descendance. Nous avons formalisé cette idée en considérant que l'indice de masculinité ou de restauration des individus dépend de nombreux facteurs additifs, un seuil dans la valeur de cet indice déterminant si l'individu est femelle ou hermaphrodite (A17).



Ce modèle a été utilisé pour réanalyser des données de croisements issus de la littérature sur 3 espèces gynodioïques, *Plantago coronopus*, *Thymus vulgaris*, *Silene vulgaris*. Il explique aussi bien les données que les modèles mendéliens proposés pour ces espèces (comprenant respectivement 5, 2 et 3 gènes de restauration) et offre une manipulation beaucoup plus simple. Il peut aussi permettre de prendre en compte les individus de morphologie intermédiaire, comme les femelles de différents types ou les individus partiellement mâle-stériles qui sont négligés dans les modèles mendéliens.

Le système gynodioïque étudié (A20) considérait un déterminisme asymétrique du sexe car d'une part c'est celui qui a été trouvé chez la betterave maritime et d'autre part le polymorphisme était réputé plus difficile à maintenir dans ce cas. Le gène de restauration avait un effet dominant sur le phénotype sexuel. Le modèle a montré que le maintien du polymorphisme nucléo-cytoplasmique n'est possible que si le coût du gène de restauration est silencieux ou constitutif. Ceci s'explique par le fait que si le coût n'affecte que les individus portant le cytotype de stérilité, le gène de restauration est neutre dans l'autre cytotype : il n'y a donc aucun lien de fréquence-dépendance entre les deux cytotypes quant à la restauration. La fréquence des hermaphrodites et des femelles s'équilibre à l'intérieur de chaque cytotype en suivant les règles du déterminisme nucléaire et le cytotype produisant le plus de graines envahit. La zone de paramètres dans laquelle on maintient la gynodioecie nucléo-cytoplasmique est plus large si le coût du gène de restauration porte sur la fonction mâle, et s'il ne s'exprime que chez les homozygotes pour ce gène – dans ce cas on crée une sorte de superdominance fonctionnelle avec des hétérozygotes restaurés pour la fonction mâle mais ne subissant pas le coût de la restauration. De plus la gynodioecie nucléo-cytoplasmique n'est possible que si les valeurs des différents paramètres se combinent de sorte à ce que la hiérarchie suivante soit respectée pour la fertilité femelle : femelle > hermaphrodite portant le cytotype fertile > hermaphrodite portant le cytotype stérile. L'équilibre obtenu peut être ponctuel ou cyclique, il est cyclique seulement lorsque le coût s'exprime de façon dominante (donc aussi chez les hétérozygotes), et plus souvent quand il porte sur la fonction mâle. L'intérêt de ces modèles est, en délimitant le champ des possibles, de prédire ce qu'on doit trouver dans les populations naturelles. On peut aussi, à l'inverse, essayer de se servir des données expérimentales pour invalider le modèle mais cela ne sera pas toujours possible car les valeurs de certains paramètres sont difficilement mesurables : les fertilités femelles des différents types

sexuels peuvent être mesurées mais ces fertilités mélangent potentiellement différents paramètres comme l'avantage femelle et l'effet du cytotype, et l'accès au coût de la restauration ne peut se faire directement et nécessite des croisements.

De façon générale, la littérature a montré que le maintien de la gynodioecie nucléo-cytoplasmique et donc d'un double polymorphisme, nucléaire et cytoplasmique, à l'intérieur d'une population nécessitait un coût de la restauration. Certaines dynamiques de métapopulations peuvent aussi expliquer ce maintien (Couvet *et al.*, 1998).

J'ai surtout cherché à savoir si la gynodioecie nucléo-cytoplasmique pouvait mener à la dioecie car la gynodioecie et la dioecie sont liées phylogénétiquement (A4) et la gynodioecie est le plus souvent nucléo-cytoplasmique mais l'idée contraire – « la gynodioecie nucléaire est instable et mène à la dioecie ; la gynodioecie nucléo-cytoplasmique est stable » – était très ancrée dans la littérature. Le ressort du problème est le suivant : l'existence de gènes de stérilité-mâle cytoplasmique favorise la présence de femelles, parfois en fortes fréquences. Cela devrait a priori favoriser un fort investissement des hermaphrodites dans la fonction mâle mais les gènes favorisant la fonction mâle seront au mieux neutres, au pire contre-sélectionnés, s'ils se trouvent dans un cytotype stérile. J'ai étudié ce problème dans différents contextes quant au déterminisme du sexe (déterminisme symétrique ou asymétrique, allocation sexuelle dépendant de formes alléliques aux locus de restauration, ou gérées par un locus modificateur indépendant) et en recherchant les conditions d'invasion d'une population gynodioïque par des mâles ou en étudiant la sélection sur l'allocation mâle des hermaphrodites (A4, A6). Les principales conclusions sont :

- lorsque le déterminisme est purement cytoplasmique (un cytotype fertile et un cytotype stérile non restauré), l'allocation à la fonction mâle est sélectionnée dans le cytotype fertile selon les conditions du déterminisme nucléaire puisque le cytotype stérile ne renvoie jamais aucun gène dans le cytotype fertile ;
- lorsque le déterminisme est nucléo-cytoplasmique, l'allocation des hermaphrodites est toujours modifiée en faveur de la fonction mâle, par comparaison aux conditions nucléaires ;
- lorsque les hermaphrodites deviennent trop mâles (ou que les mâles envahissent), on a tendance à perdre le polymorphisme cytoplasmique dans le cas d'un déterminisme asymétrique, on peut le garder, même si le système devient dioïque, dans le cas d'un déterminisme symétrique (A2).

Le passage à la dioecie fragilise donc le maintien du polymorphisme cytoplasmique, ce qui implique qu'on peut éventuellement ne pas retrouver de traces de ce polymorphisme dans des espèces dioïques même si l'espèce ancêtre possédait un déterminisme nucléo-cytoplasmique du sexe.

1.2. Les cas particuliers de la distylie et du polymorphisme de longueur de style

Les espèces distyles sont caractérisées par la présence de deux catégories d'hermaphrodites – appelées morphes – différant de façon réciproque pour la hauteur de leurs organes mâles et femelles (voir tableau 1). Ces morphes sont gouvernés par un super gène, nous sommes donc là encore dans le cas de deux phénotypes correspondant à deux catégories génotypiques. De plus, les espèces distyles présentent en général un système d'incompatibilité intramorphe : un individu d'un morphe est incompatible avec tous les individus du même morphe, mais compatible avec tous les individus de l'autre morphe. L'évolution d'un tel système est complexe et deux théories s'affrontent : le système d'incompatibilité aurait évolué en premier pour éviter l'autofécondation, suivi par une séparation spatiale des organes pour accroître l'efficacité du transfert de pollen entre

les classes d'incompatibilité (Charlesworth & Charlesworth, 1979) ou bien la séparation spatiale des organes aurait évoluée en premier (Lloyd & Webb, 1992). On comprend bien en revanche qu'une fois mis en place le système est très stable puisque là encore on a une fréquence-dépendance automatique, le morphe le plus rare étant meilleur mâle car pouvant polliniser plus d'individus, et éventuellement meilleur femelle si le pollen est limitant de la fécondité femelle.

Il existe aussi des cas de distylie sans système d'incompatibilité, c'est notamment le cas chez les narcisses. Dans ces cas la fréquence-dépendance n'existe que si les transferts de pollen se font préférentiellement d'un morphe vers l'autre. Il est donc crucial de pouvoir tester cette hypothèse de transferts préférentiels et nous avons développé une méthode pour les estimer dans le cas où la taille du pollen diffère partiellement entre les deux morphes, ce qui est généralement le cas dans les espèces distyles (thèse de Thierry Pailler - A13). Le problème est identique pour le polymorphisme de longueur de style, dans lequel les morphes ne diffèrent que pour la hauteur des organes femelles (tableau 1) et ne présentent pas de classes d'incompatibilité. Ce système de reproduction est rare en général et il est proposé que ce soit un stade intermédiaire dans l'évolution de la distylie. Il est bien représenté dans le genre *Narcissus* et le cas de *Narcissus assoanus* a été étudié par Adeline Cesaro pendant sa thèse. Chez cette espèce le pollen des deux morphes est de même taille et elle a quantifié l'efficacité des transferts de pollen entre les différents morphes par des manipulations de morphe-ratio (Cesaro & Thompson, 2004). Elle a également quantifié les interférences négatives entre fonctions mâle et femelle au sein d'une plante (appelées auto-interférences : gêne entre organes au niveau spatial, encombrement du style par l'autopollen, perte d'ovule par autoincompatibilité tardive…) (A16). Ces données ont ensuite été intégrées à des équations que nous avons développées pour décrire l'évolution de la fréquence des morphes. Les deux morphes peuvent être maintenus si l'auto-interférence et l'autofécondation sont évitées, et si le pollen est limitant de la fertilité femelle. Le modèle montre également que des changements de morphe-ratio peuvent être expliqués par une variation de la limitation de pollen, même si celle-ci est d'égale intensité dans les deux morphes : bien sûr des changements qualitatifs du cortège de pollinisateur favorisant l'un ou l'autre morphe auront des conséquences sur leurs fréquences mais des changements uniquement quantitatifs aussi (thèse d'Adeline Cesaro).

1.3. Coexistence de deux phénotypes sexuels correspondant à une plasticité des individus

Des facteurs environnementaux influencent parfois l'expression du sexe, même chez des espèces décrites comme dioïques (revue dans Korpelainen, 1998) mais le déterminisme reste pour une grande part génétique et on parle de « labilité du sexe » plutôt que de déterminisme environnemental du sexe. Les exemples les mieux décrits de plasticité du phénotype sexuel concernent les plantes monoïques (fleurs femelles et mâles sur un même pied) et les plantes andromonoïques (fleurs hermaphrodites et mâles sur un même pied) dont le pourcentage de fleurs mâles peut varier en fonction de la taille de la plante (Thomson *et al.*, 1989) ou du niveau de ressources (Dorken & Barrett**,** 2004) jusqu'à faire apparaître des individus seulement mâles dans les populations. Mais il ne s'agit pas de deux phénotypes clairement séparés et je reparlerai au chapitre suivant de ce problème de catégories séparées ou non. On observe en général plus de fleurs mâles dans les milieux défavorables. L'explication abondamment proposée dans la littérature est que la fonction femelle est coûteuse et que par conséquent les individus se contenteraient d'être mâle dans de mauvaises conditions, pour devenir hermaphrodite ou même femelle dans de meilleures conditions. Nous verrons dans un chapitre ultérieur (*2.4.1*) qu'il faut en fait des conditions plus précises pour qu'une variation environnementale favorise un changement de stratégie sexuelle.

Modèles phénotypiques Encadré 3

Exemple de l'influence de la limitation de pollen sur l'évolution du système de reproduction

Méthode

La méthode utilisée repose sur la théorie des jeux ou l'analyse d'invasion par un mutant rare. On écrit la valeur sélective de chaque stratégie ; celle-ci dépend des fréquences des différentes stratégies dans la population. Une stratégie sera considérée comme évolutivement stable si, lorsqu'elle est prépondérante («stratégie résidente »), aucune autre stratégie possible (« stratégie mutante ») ne peut l'envahir, c'est-à-dire lorsque toutes les autres stratégies ont une valeur sélective inférieure.

Dans le cas des systèmes de reproduction, les stratégies - ou phénotypes - possibles sont femelle, mâle, hermaphrodite, qui se combinent en hermaphrodisme, gynodioecie, androdioecie, dioecie ou trioecie. Pour savoir par exemple si la dioecie est stable, on considère qu'il n'y a que des mâles et des femelles dans la population. A l'équilibre ces deux phénotypes doivent avoir la même valeur sélective, ce qui permet d'obtenir conjointement leurs fréquences et leurs valeurs sélectives. On calcule avec les fréquences obtenues la valeur sélective d'un rare hermaphrodite et on compare cette valeur à celle des mâles et femelles.

Modèle avec limitation par les pollinisateurs de l'autofécondation et de l'allofécondation

Les paramètres sont :

α : production de pollen des hermaphrodites relativement aux mâles

β : production de graines des hermaphrodites relativement aux femelles (en absence de limitation de la fertilité femelle par le pollen et de dépression de consanguinité)

p_s, p_c : probabilité qu'une fleur soit assez visitée pour produire des graines autofécondées (s), des graines allofécondées (c)

s : taux d'autofécondation des hermaphrodites « a priori » - le taux réalisé (s_a) dépend de l'intensité de la limitation de pollen et est égal à : $sp_s/(sp_s + (1-s)p_c)$

d : dépression de consanguinité

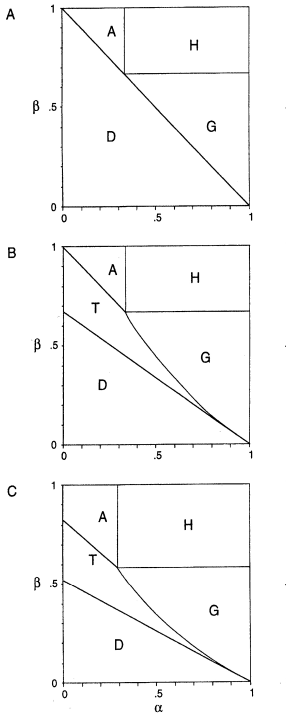
Avec f, m, h : fréquences des femelles, mâles et hermaphrodites dans la population, les valeurs sélectives relatives des différents types sexuels sont :

$$W_f = p_c$$

$$W_h = \beta\left[(1-s)p_c + 2s(1-d)p_s\right] + \frac{\alpha\left[fp_c + h\beta(1-s)p_c\right]}{m + \alpha h}$$

$$W_m = \frac{fp_c + h\beta(1-s)p_c}{m + \alpha h}$$

On peut voir que seul le ratio p_c/p_s intervient, il sera dénoté p_f.



La figure ci-dessus présente le système de reproduction stable dans les situations suivantes :

A- il y a assez de pollinisateurs et de pollen donc pas de limitation de la fertilité femelle par la pollinisation (p_f =1) ;

B- en absence de femelle, l'autofécondation est aussi limitée que l'allofécondation par l'activité des pollinisateurs ; lorsque le taux de femelle augmente, le manque de pollen limite plus fortement l'allofécondation que l'autofécondation (p_f=1-f^x) ;

C- l'allofécondation est toujours plus limitée par la pollinisation que l'autofécondation (p_f=a(1-f^x) avec a<1) ;

Pour ces trois cas : x = 1, s = 0.5 et d = 0.5 ;

H : Hermaphrodisme - D : Dioecie - G : Gynodioecie - A : Androdioecie - T : Trioecie

2. Coexistence de plus de deux phénotypes sexuels

Dans la plupart des espèces présentant un système polymorphe plus complexe que la dioecie se pose encore plus cruellement la question du lien entre phénotype et génotype. Dans la plupart des cas on ne connaît rien sur le déterminisme du sexe. La définition même des catégories phénotypiques peut poser problème. En effet, et comme on le verra dans les études empiriques détaillées plus loin, dans la plupart des cas décrits comme « trioïques » ou « subdioïques » les femelles sont définies par le faible développement des étamines et l'absence de pollen viable mais les mâles ne sont souvent définis qu'a posteriori - un mâle n'étant qu'un hermaphrodite qui n'a pas fructifié – et il est difficile d'affirmer que les mâles et les hermaphrodites forment deux catégories séparées, d'autant plus que l'expression sexuelle des individus n'est pas toujours stable entre saisons.

2.1 Coexistence de trois phénotypes sexuels correspondant à trois catégories génotypiques – la théorie

Certains modèles d'évolution des systèmes de reproduction – cherchant les conditions du passage de l'hermaphrodisme à la dioecie – peuvent aussi donner des conditions pour lesquelles les trois types sexuels (femelle, hermaphrodite, mâle, tous d'expression non plastique ou stable) peuvent coexister. Ce système, la « trioecie vraie », est rare et les conditions pour l'obtenir sont détaillées ci-dessous.

2.1.1 Les modèles phénotypiques

Les résultats des modèles phénotypiques - valides lorsque le déterminisme génétique du sexe est nucléaire et sans contrainte – ne font pas apparaître la trioecie comme système de reproduction (voir encadré 1). En fait la coexistence des trois types d'individus n'est possible que si les hermaphrodites « se situent sur la diagonale » dans l'espace des fertilités, c'est-à-dire si la somme de leurs fertilités mâle et femelle relatives vaut exactement un. Dans le formalisme des fitness-sets cela implique que les deux fonctions sexuelles ont des courbes de gain en fonction de l'investissement linéaires. Des valeurs ajustées à ce point ne sont pas biologiquement réalistes. De plus même si cela garantit que tous les phénotypes sont équivalents en population infinie, les fréquences dérivent en population finie et la trioecie n'est pas stable.

Des observations sur une espèce présentant des populations trioïques (voir paragraphe *2.2*) nous ont conduit à compléter les modèles phénotypiques existants pour inclure l'autofécondation et la limitation de la fertilité femelle par un défaut de pollinisation (A8). Certaines espèces, comme celle étudiée, ne peuvent faire d'autopollinisation, c'est-à-dire d'autofécondation sans intervention d'un agent. L'autofécondation peut donc être limitée par l'activité des pollinisateurs. L'allofécondation sera néanmoins souvent plus limitée par cette activité et éventuellement d'autant plus qu'il y a de non-producteurs de pollen (femelles) dans la population. Ces différentes situations sont comparées dans l'encadré 3 ci-contre.

Les frontières entre les différents systèmes de reproduction sont affectées par la limitation de la fertilité par la pollinisation : celle-ci augmente l'intervalle de paramètres dans lequel on trouve des hermaphrodites, même lorsque la dépression de consanguinité est forte dès lors que l'allofécondation est plus limitée que l'autofécondation. Si la limitation par la pollinisation est plus sévère pour l'allofécondation que pour l'autofécondation et que cette différence dépend de la fréquence des femelles, il existe une zone de paramètres dans laquelle la trioecie est le système de reproduction stable (figure de l'encadré 3).

Modèles génétiques Encadré 4

Exemple de l'influence du déterminisme génétique sur l'évolution du système de reproduction

Méthode

A partir du déterminisme du sexe et des autres hypothèses du modèle, on connaît les productions de gamètes mâles et femelles des différents génotypes. Les fertilités réalisées dépendent bien sûr des fréquences des différents génotypes dans la population et conditionnent l'évolution de ces fréquences. L'évolution des fréquences génotypiques est donc décrite par un système d'équations de récurrence qui sera itéré jusqu'à obtention d'un équilibre.

Dans le cas de la recherche de l'influence du déterminisme nucléo-cytoplasmique du sexe sur l'allocation à la fonction mâle des hermaphrodites, on peut procéder de plusieurs façons :

- soit on considère un hermaphrodite aux caractéristiques fixées et on le met en compétition avec des mâles, c'est-à-dire qu'on introduit une mutation de stérilité femelle totale et on observe son devenir. On effectue cette démarche pour l'ensemble des hermaphrodites possibles (cf. figure ci-dessous).
- soit on choisit un fitness-set (ensemble des possibilités de fertilités pour un individu, voir encadré 1) et on calcule d'abord quelle est l'allocation aux fonctions mâle et femelle évolutivement stable pour des hermaphrodites seuls ; puis on recherche l'équilibre gynodioïque avec cet hermaphrodite. On peut ensuite soit examiner le devenir d'une mutation complètement femelle-stérile comme précédemment, soit introduire une mutation qui modifie l'allocation sexuelle des hermaphrodites. Dans ce dernier cas, on conserve l'allèle « gagnant » et de proche en proche, on détermine la nouvelle allocation sexuelle évolutivement stable.

Modèle avec déterminisme nucléo-cytoplasmique symétrique du sexe (voir encadré 2)

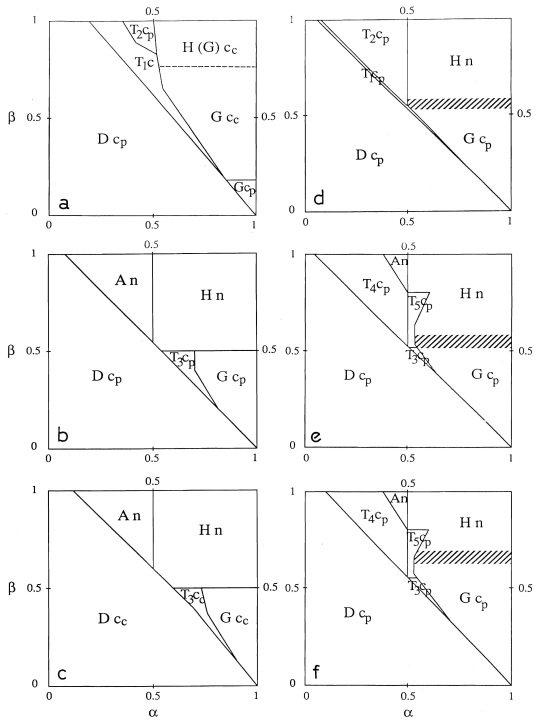
Les paramètres sont :

α : production de pollen des hermaphrodites relativement aux mâles,

β : production de graines des hermaphrodites relativement aux femelles,

d_m, d_f : coût de la restauration sur la fonction mâle, sur la fonction femelle (voir encadré 2).

La figure ci-contre montre le système de reproduction en fonction des caractéristiques de l'hermaphrodite (α, β) pour un coût de la restauration fort (a-b-c) ou faible (d-e-f), portant sur la fonction mâle (a-d), femelle (b-e) ou les deux (c-f).



H : Hermaphrodisme
D : Dioecie
G : Gynodioecie
A : Androdioecie
T : Trioecie (les chiffres correspondent à différentes répartitions des types sexuels dans les cytotypes– voir texte principal)

c : le polymorphisme nucléo-cytoplasmique est maintenu (l'équilibre est un point c_p ou un cycle c_c)
n : perte du polymorphisme cytoplasmique

Ces résultats sont à comparer aux résultats lorsque le déterminisme du sexe est nucléaire (encadré 1).

2.1.2 Les modèles génétiques

Le modèle d'évolution de la dioecie de Charlesworth et Charlesworth (1978) fait intervenir l'augmentation en fréquence d'une mutation mâle-stérile (créant des femelles) dans une population d'hermaphrodites, puis d'une mutation femelle-stérile (créant des mâles). Ces deux mutations sont à des locus différents et, si ces locus ne sont pas totalement liés, des hermaphrodites, ainsi que de plus rares individus double-stériles, perdureront dans les populations, même si les conditions sont telles que c'est la dioecie qui est sélectionnée. Cette situation n'est néanmoins pas stable à long terme puisqu'une plus forte liaison entre ces deux locus du sexe est sélectionnée; c'est cette sélection qui est à la base de la théorie sur la formation des chromosomes sexuels.

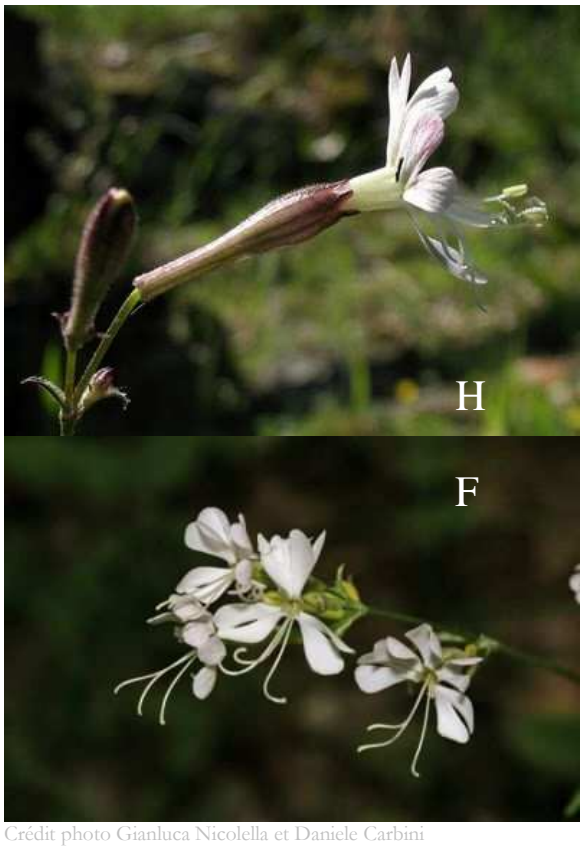
Un modèle avec un déterminisme du sexe à un locus-deux allèles prédit une trioecie stable lorsque les unisexués sont sélectionnés mais que l'hermaphrodite est le génotype hétérozygote (Gregorius, Ross & Gillet, 1983). Là encore c'est le déterminisme qui donne la contrainte et qui est directement responsable du maintien de trois types. Ce déterminisme peut de plus être jugé irréaliste et n'a, à ma connaissance, jamais été observé.

Des cas de trioecie apparaissent dans des modèles avec un déterminisme nucléo-cytoplasmique du sexe (Gregorius & Ross, 1987; A4 ; A6 : Schultz, 1994). Le cas développé par Gregorius et Ross (1987) est à nouveau très particulier et force le maintien des trois types : l'allèle qui détermine le fait d'être mâle dans un cytotype détermine le fait d'être hermaphrodite dans l'autre. Dans les modèles à déterminisme symétrique (deux cytotypes stériles - A6) ou asymétrique (un cytotype stérile, un cytotype fertile – A4) du sexe, les calculs font apparaître que dans une certaine zone de fertilité relative les mâles augmentent en fréquence mais ne remplacent pas les hermaphrodites. Ces trioecies sont néanmoins plus ou moins stables selon les valeurs de paramètres et surtout selon les détails du déterminisme du sexe. Ainsi par exemple dans le cas du déterminisme symétrique, lorsque le phénotype mâle est déterminé par un gène modificateur à un locus différent des locus de restauration, des cas de trioecie globalement stable sont obtenus : le sex-ratio à l'équilibre sera le même quelles que soient les fréquences de départ et notamment que les mâles ou les hermaphrodites soient le phénotype rare au début du calcul. Lorsque le phénotype mâle est déterminé par une forme allélique du gène de restauration (donc spécifique d'un cytotype), on obtient généralement des cas de trioecie localement stable : pour certaines fertilités, le premier « restaurateur mâle » introduit envahit son compartiment cytoplasmique qui devient alors dioïque mais le restaurateur mâle spécifique du second cytotype ne peut alors plus envahir. En revanche, une fois cet équilibre obtenu, de faibles perturbations des fréquences n'auront pas de conséquence. La trioecie est alors le système de reproduction stable mais tous les type sexuels ne sont pas présents dans tous les contextes cytoplasmiques. D'autre part la trioecie sera plus facilement obtenue si les mâles apparaissent rapidement dans les populations, avant que l'allocation des hermaphrodites ne soit ajustée à la nouvelle situation ; autrement dit le système de reproduction obtenu dépendra de l'ordre d'apparition des différents types de mutation : stérilité femelle totale ou partielle.

Pachycereus pringlei
– le cardon

Senecio inaequidens
– le séneçon du Cap

Silene italica
– le silene d'Italie



Crédit photo Gianluca Nicolella et Daniele Carbini

Silene acaulis
– le silene acaule

Figure 1 : **Illustration des principales espèces étudiées.**

2.2 Le cactus Pachycereus pringlei – un vrai cas de trioecie

La différence entre trioecie et subdioecie pourrait être seulement une question de fréquence des hermaphrodites, mais ce qui distingue encore plus nettement le cas de trioecie dont je vais parler ici des cas de subdioecie de la littérature est que les catégories sexuelles se distinguent a priori, c'est-à-dire avant la fructification, sur des critères morphologiques discrets. Comme souvent les femelles se distinguent des hermaphrodites par leur absence de pollen mais de plus les mâles se distinguent des hermaphrodites par leur absence totale d'ovule. Toutes les fleurs d'un même individu sont d'un même type sexuel et les hermaphrodites sont en fortes fréquences dans les populations, la trioecie est donc clairement établie.

2.2.1 Description de l'espèce

Le cardon, *Pachycereus pringlei* (figure 1), est un cactus colonne géant qui vit dans le désert du Sonora et en Basse Californie (Mexique). Il est autotétraploïde, vit plus de 100 ans. Chaque fleur ne reste ouverte qu'une seule nuit et est visitée essentiellement la nuit par les chauves-souris et dans une moindre mesure à l'aube par des oiseaux.

Les fleurs ont toutes une apparence hermaphrodite. Les étamines des fleurs femelles sont plus petites et plus foncées, elles ne contiennent pas de pollen. Les styles des fleurs mâles sont normalement développés mais les ovaires ne contiennent pas d'ovules. Chaque individu ne présente qu'un type de fleur et le type sexuel des individus est stable sur les 6 ans d'étude. C'est peu dans la vie d'un tel cactus mais un autre argument en faveur d'une stabilité du sexe est que les types sexuels ne diffèrent pas pour la taille.

Une population trioïque a été étudiée en détail et a donné les résultats suivants (A5). La proportion de fleur donnant des fruits est limitée par le pollen chez les femelles mais pas chez les hermaphrodites : ces proportions sont égales en pollinisation libre (35%), la production de fruit des hermaphrodites n'augmente pas si on supplémente en pollen mais celle des femelles si. Les femelles et les hermaphrodites ne diffèrent pas pour la production de graines par fruit. La quantité de pollen par fleur est identique chez les mâles et les hermaphrodites. En revanche le nombre de fleurs produites diffère selon le sexe : M>F>H. Le tout se solde par un avantage femelle de 1.62 (nombre de graines produites par femelle/ nombre de graines produites par hermaphrodite) et une avantage mâle de 1.52 (nombre de grains de pollen produits par mâle/ nombre de grains de pollen produits par hermaphrodite). Les hermaphrodites présentent dans cette population un taux d'autofécondation de 65% et la dépression de consanguinité est faible dans les premiers stades (nombre de graines et germination). L'autofécondation nécessite la visite de pollinisateurs (8% seulement de production de fruit pour les fleurs ensachées).

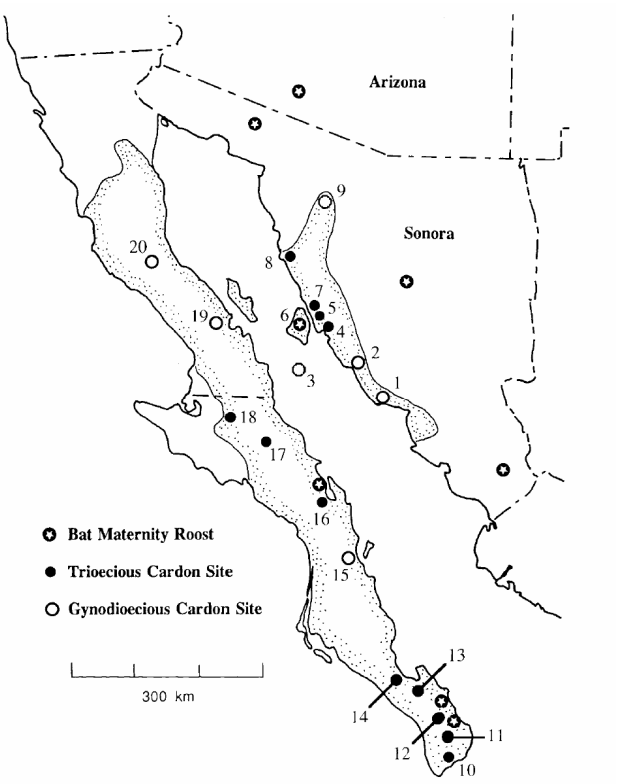


Figure 2 : **Localisation géographique des populations gynodioïques (○) et trioïques (●) de *Pachycereus pringlei*** sur l'ensemble de son aire de répartition. Les grands sites de reproduction des chauves-souris sont aussi localisées (✪).

Site[a]	*n*	%Males	%Females	% Hermaphrodites	% Neuters
1. San Carlos, Son.	43	0	30.2	69.8	0
2. Tastiota Junction, Son.	48	0	33.3	66.7	0
3. Isla San Pedro Martir, Son.	93	0	46.2	53.8	0
4. Bahia Kino, Son.	211	28.4	41.7	28.0	1.9
5. Madrugada, Son.	47	42.6	38.3	19.1	0
6. Isla Tiburon, Son.	5	40.0	20.0	40.0	0
7. Punta Chueca, Son.	71	22.5	31.0	46.5	0
8. Puerto Libertad, Son.	59	35.6	39.0	25.4	0
9. Caborca, Son.	29	3.4	40.7	55.6	0
10. Cabo San Lucas, BCS	44	53.7	36.6	9.8	0
11. Santiago, BCS	16	68.7	31.3	0	0
12. Buena Vista, BCS	43	53.5	27.9	16.3	2.3
13. S of La Paz, BCS	20	75.0	20.0	5.0	0
14. W of La Paz, BCS	32	50.0	46.8	3.1	0
15. Loreto, BCS	37	0	29.7	70.2	0
16. Playa Cocos, BCS	33	30.3	36.4	33.3	0
17. W of Santa Rosalia, BCS	35	31.4	42.9	20.0	5.7
18. SE of Guerrero Negro, BCS	25	52.0	24.0	16.0	8.0
19. W of Bahia de Los Angeles, BCN	42	0	16.7	83.3	0
20. Catavina, BCN	34	0	44.1	55.9	0

[a] Numbers refer to the sites in Fig. 1. Except at Bahia Kino (site 4), data represent the frequencies observed on one day and may not represent true frequencies because hermaphrodites begin to flower somewhat later in the blooming season (by 1–2 weeks) than males and females (Fleming *et al*., 1994). Populations in Sonora were surveyed in mid-flowering season. Those in Baja California were surveyed early in the flowering season when the frequency of hermaphrodites may have been underestimated. Son. = Sonora, BCS = Baja California Sur, BCN = Baja California Norte.

Tableau 2 : Fréquences des types sexuels par population.

Cette espèce présente en fait des populations dioïques et des populations gynodioïques (figure 2 et tableau 2 ci-contre, A10). L'absence de mâles dans certaines populations ne semble pas être due à la non apparition de la mutation de stérilité femelle car les analyses génétiques concluent à un fort flux de gènes entre populations. Les populations trioïques sont plus proches des caves de chauves-souris et les chauves-souris arrivent plus tôt dans ces populations. En revanche nous n'avons pas trouvé de différence entre les deux types de populations pour la probabilité pour une fleur d'être visitée au moins une fois. Le nombre de visite n'a pas pu être mesuré.

2.2.2 Les explications possibles

Pratiquement toutes les autres espèces de cactus documentées sont diploïdes et hermaphrodites. L'explication la plus logique serait que la trioecie observée chez le cardon soit un stade transitoire dans l'évolution de l'hermaphrodisme vers la dioecie. Cette hypothèse parait d'autant plus naturelle que la polyploïdisation cause parfois la rupture du système d'auto-incompatibilité (Mable, 2004) et l'unisexualité peut alors être sélectionnée pour éviter l'autofécondation (Miller & Venable, 2000). Mais d'autre part l'autofécondation diminue moins fortement l'hétérozygotie chez les polyploïdes et la dépression de consanguinité pourrait donc être moins importante. Le résultat net n'est donc pas évident. Il existe des exemples dans les deux sens (diploïde hermaphrodite -tétraploïde dioïque et l'inverse comme par exemple chez le cactus *Echinocereus*) et la polyploïdisation peut aussi perturber le déterminisme génétique du sexe chez les dioïques et donc ramener à la bisexualité.

Dans le cas d'une évolution vers la dioecie, les hermaphrodites et les neutres (double-stériles) perdureraient dans les populations à cause d'une liaison incomplète entre les locus de stérilité mâle et femelle (Charlesworth & Charlesworth, 1978). Mais les estimations des fertilités des différents types sexuels ne correspondent pas avec un stade de transition vers la dioecie (sous hypothèse de déterminisme nucléaire) car les hermaphrodites se reproduisent mieux que les unisexués lorsqu'on combine leurs fertilités mâle et femelle. Les données suggéreraient plutôt une réversion vers l'hermaphrodisme mais aucune espèce proche n'est dioïque.

Des modèles ont montré que la trioecie est possible si le déterminisme n'est pas seulement nucléaire (voir précédemment) mais nous ne pouvions éclaircir le déterminisme du sexe dans cette espèce par la méthode classique des croisements puisque la floraison commence après l'âge de 50 ans. La génomique pourrait maintenant aider à résoudre ce problème (en cherchant des gènes cytoplasmiques de stérilité par exemple).

L'hypothèse que nous avons privilégiée est que la coexistence des trois types d'individus serait due à une influence des pollinisateurs sur le succès reproducteur. Le fait que les populations trioïques soient les plus près des grands sites de reproduction des chauves-souris, qui sont leur principal pollinisateur, est en faveur de cette hypothèse. Les mâles alors seraient présents dans les populations où la pollinisation est la plus importante et ce en basse Californie comme dans le désert du Sonora. Nous avons montré de plus que la trioecie peut être stable si la limitation par le pollen est liée à la fréquence des femelles dans les populations (voir *2.1.1*). Il reste néanmoins qu'avec les fertilités obtenues, pourtant mesurées dans une population trioïque, le système devrait plutôt être gynodioïque (A10).

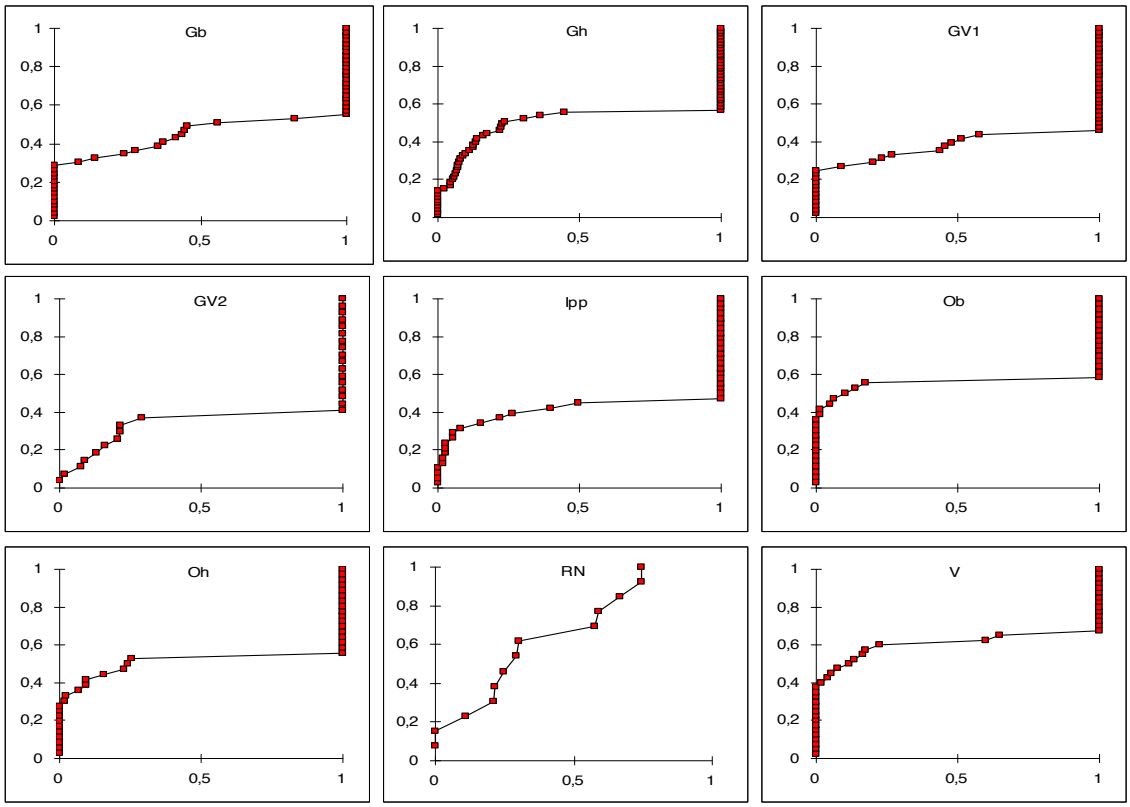
La subdioecie Encadré 5

Genre phénotypique des individus de différentes populations de la sous-espèce Silene acaulis cenisia

Le genre phénotypique a été défini par Lloyd (1980) et est couramment utilisé dans la caractérisation des systèmes polymorphes. Il n'est pas le genre réalisé puisque seules des analyses de paternité peuvent atteindre ce dernier mais il donne un indice du genre potentiel en tenant compte du phénotype sexuel des autres individus de la population. La « féminité phénotypique » d'un individu (G_i) est le rapport entre sa production de fruits (d_i) et la somme de sa production de fruits et de sa contribution potentielle comme mâle ; cette dernière est égale à son nombre de fleurs staminés (l_i) multiplié par la somme des fruits de la population et divisé par le nombre total de fleurs staminés de la population.

$$G_i = \frac{d_i}{d_i + l_i.E}, \quad \text{avec } E = \frac{\sum_j d_j}{\sum_j l_j}$$

Cet indice permet de montrer la variabilité entre individus et entre populations. La figure ci-dessous représente le % cumulé d'individus en fonction de leur genre phénotypique dans 9 populations.



Je trouve néanmoins que cet indice peut donner des idées fausses car les hermaphrodites sont « repoussés » vers le pôle mâle par la présence de femelles. C'est exact puisqu'ils se reproduiront en moyenne plus par la fonction mâle en présence de femelles mais cela a pour effet i) de tasser la variabilité / variabilité sur le fruit-set et surtout ii) en éloignant les hermaphrodites des femelles, laisser croire que leur fonction femelle est minime. Il ne faut donc pas confondre cet indice avec l'avantage femelle.

Par exemple, dans une population composée à 50:50 d'H et de F produisant tous autant de fleurs, avec un fruit-set moyen des F = 80% et un fruit-set moyen des H = 20% :
les F ont par construction un indice de 1 ;
un H dont le fruit-set est 80% n'aura qu'un indice de 0.44 ;
un H dont le fruit-set est 20% aura un indice de 0.17.

2.3. La subdioecie et la gynomonoecie : deux ou trois catégories génotypiques ?

Le problème de savoir si on a à faire à des phénotypes correspondant à des génotypes différents ou si la plasticité explique en partie la variation phénotypique, se pose dans ce qui a été appelé la subdioecie : présence de femelles, de mâles et soit d'un très faible pourcentage d'hermaphrodites, soit, le plus souvent, d'hermaphrodites produisant peu de fruits et dans ce qui a été appelé la gynomonoecie ou gynomono-dioecie : présence de femelles, d'hermaphrodites et de plantes gynomonoïques.

J'ai étudié ces deux systèmes dans des espèces du genre *Silene*. J'ai à l'origine choisi ce genre car les espèces gynodioïques à déterminisme nucléo-cytoplasmique du sexe les mieux étudiées à l'époque – *Beta maritima*, *Thymus vulgaris* et *Plantago lanceolata* – ne possédaient pas d'espèces dioïques congénériques. Le genre *Silene* présente une grande variété de système de reproduction, avec un système ancestral gynodioïque (ou gynomono-dioïque) et plusieurs évènements d'évolution vers la dioecie ou l'hermaphrodisme (A9). Ce genre est de plus devenu un genre modèle pour l'étude de l'évolution des chromosomes sexuels et d'autres questions dont la plupart liées à la reproduction (Bernasconi *et al.*, 2009).

2.3.1 La subdioecie – Silene acaulis et Antirhea borbonica

Silene acaulis a été décrite comme gynodioïque aux Etats-Unis, comme trioïque ou dioïque selon les sous-espèces en Europe. En France, Bock (1976) décrit la sous-espèce *escapa* comme dioïque, et la sous espèce *cenisia* comme trioïque sur la base de la morphologie florale : les fleurs staminés d'*escapa* montrent toutes un style réduit, les fleurs staminés de *cenisia* montrent un style réduit (mâles morphologiques) ou développé (hermaphrodites morphologiques).

Plusieurs populations des deux sous-espèces ont été étudiées dans le but de vérifier l'adéquation entre le type sexuel morphologique et celui réalisé. Quelques mâles d'*escapa* ont donné des fruits mais la différence de système de reproduction entre les deux sous-espèces est confirmée : le ratio entre la production de fruit des femelles et des individus staminés vaut 10 chez *cenisia* et 900 chez *escapa*. Le classement a priori sur la morphologie ne reflète pas précisément les potentialités des plantes staminées de *cenisia* puisque deux tiers des hermaphrodites morphologiques et la moitié des mâles morphologiques donnent des fruits. Les individus classés a priori comme hermaphrodites montrent une fonction femelle variable (figure encadré 5) mais se distinguent néanmoins par des nombres de fleurs, de fruits, de fleurs et de fruits rapportés à la taille et un fruit-set (ratio fruits/fleurs) significativement plus élevés que les individus classés a priori comme mâles. Les hermaphrodites et les mâles morphologiques sont donc différents mais semblent être les deux extrema d'un continuum plutôt que deux catégories distinctes. Le fait que les hermaphrodites morphologiques soient plus vigoureux pose aussi la question de savoir si le phénotype sexuel ne serait pas influencé (avant le stade de fructification) pas la vigueur de la plante ou un environnement favorable.

J'illustrerai la subdioecie par un autre exemple, que je n'ai pas étudié personnellement mais qui a motivé les développements théoriques présentés en *2.4.1. Antirhea borbonica* (Rubiaceae) est une espèce ligneuse endémique des îles de la Réunion et Maurice. Cette espèce est pionnière des coulées de lave mais persiste lors de la succession. On la trouve également dans de très vieilles forêts, hors de la zone d'activité volcanique. Cette espèce a été étudiée par Isabelle Litrico pendant sa thèse. Certains individus portent exclusivement des fleurs dont les étamines, réduites,

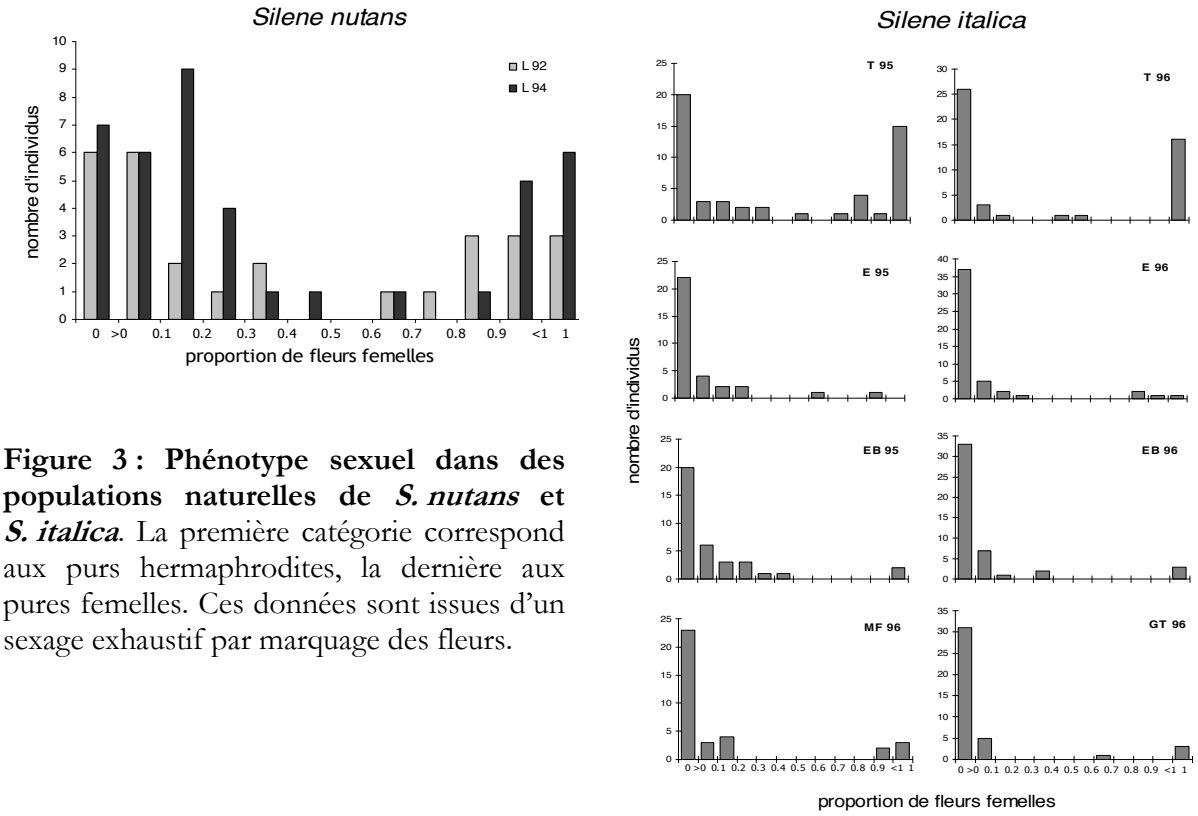


Figure 3 : Phénotype sexuel dans des populations naturelles de *S. nutans* et *S. italica*. La première catégorie correspond aux purs hermaphrodites, la dernière aux pures femelles. Ces données sont issues d'un sexage exhaustif par marquage des fleurs.

Une considération sur la gynomonoecie Encadré 6

Influence des gynomonoïques sur l'avantage femelle

On a vu qu'un paramètre crucial pour l'évolution des systèmes de reproduction est l'avantage femelle, c'est-à-dire la fertilité des femelles relative à la fertilité femelle des hermaphrodites.
L'existence de gynomonoïques peut modifier ce calcul. Il faudrait connaître le déterminisme précis du sexe mais les gynomonoïques étant au moins en partie restaurés, on a choisi ici de les regrouper avec la catégorie hermaphrodite.
D'autre part, dans les modèles de gynodioecie, cet avantage correspond aux rapports de fertilité d'individus ne différant que pour le sexe et possédant pour le reste le même contexte génomique et notamment cytoplasmique. Si, et c'est ce qui est d'ailleurs attendu, les hermaphrodites et les femelles portent en moyenne des cytotypes différents, la mesure de l'avantage femelle en populations mélangera les effets du sexe et du cytotype et ne reflétera pas les paramètres des modèles.

Le tableau ci dessous présente, pour différentes familles de *S.italica*, l'avantage femelle pour le fruit-set, le nombre total de fruits et le poids total de graines. Cet avantage est calculé sans ou avec les gynomonoïques, en tenant compte du % d'hermaphrodites et de gynomonoïques dans chaque famille.

Famille	Fruit-set		Nombre de fruits		Poids des graines	
	F / H	F / (H+G)	F / H	F / (H+G)	F / H	F / (H+G)
E	1.33	1.29	1.00	0.96	1.67	1.56
I	1.50	1.49	1.46	1.51		
K	1.62	1.53	1.42	1.31		
P	1.75	1.64	1.32	1.30		
S	1.24	1.18	0.97	0.99		
Y	1.22	1.14	1.17	1.05		
V	1.22	1.24	1.21	1.19	1.28	1.14
Z	1.23	1.20	1.22	1.17		

Ces données montrent que la variation de l'avantage femelle est plus forte entre famille que la variation entre les estimations avec ou sans les gynomonoïques.

ne produisent pas de pollen: ce sont donc clairement des femelles. Certains individus produisant du pollen ont la capacité de fructifier, les individus pollinifères sont donc mâles ou hermaphrodites; tous les individus pollinifères possèdent des ovules et l'examen morphologique seul ne permet pas leur classification a priori. L'étude de la reproduction dans différentes populations a montré que les individus pollinifères des populations les plus vieilles, que ces populations soient dans ou hors enclos (zone d'activité volcanique), fructifiaient plus que ceux des populations plus jeunes (toutes dans l'enclos). En comparant plusieurs années, on constate que ce ne sont pas toujours les mêmes individus pollinifères qui fructifient, on ne peut donc pas séparer deux catégories. De même, le pourcentage d'arbres qui donnent au moins un fruit augmente avec le fruit-set des individus pollinifères dans la population. I. Litrico a manipulé directement le niveau de ressources et après deux ans de supplémentation presque tous les mâles fructifient (Litrico *et al.*, 2005). Le sexe des plantes montre donc une composante plastique en fonction des ressources.

2.3.2 La gynodioecie-gynomonoecie – Silene nutans et S. italica

Un cas moins classique de polymorphisme à plus de deux phénotypes est constitué par la présence d'individus gynomonoïques, c'est-à-dire portant des fleurs femelles et hermaphrodites, aux cotés d'individus portant uniquement des fleurs femelles ou uniquement des fleurs hermaphrodites. Ces individus mixtes ont souvent été négligés (regroupés avec une autre catégorie, éliminés de l'échantillon ou probablement parfois même passés sous silence) bien qu'ils aient été observés dans de nombreuses espèces gynodioïques et qu'ils soient parfois aussi fréquents dans les populations que les femelles (revue dans Koelewijn & van Damme, 1996). Ils sont présents dans plusieurs espèces de *Silene* (A9) et notamment dans les deux espèces sur lesquelles je me suis penchée, *S. italica* et *S. nutans*. Il a été choisi d'étudier ce phénotype chez *S. nutans* lors du DEA puis de la thèse de Christine Desfeux. Les questions soulevées par ces individus intermédiaires sont nombreuses. Représentent-ils une stratégie sexuelle ou une contrainte liée au déterminisme génétique du sexe? Sont-ils déterminés génétiquement et si oui comment ? Les différentes hypothèses pouvant être une restauration partielle, liée à peu de gènes ; une restauration quantitative ; l'expression d'une hétéroplasmie (coexistence à l'intérieur d'un individu de plusieurs types cytoplasmiques différant pour leur stérilité). Quelle peut être leur influence sur l'évolution des systèmes gynodioïques (exemple dans l'encadré 6)?

S'attaquer à ces questions suppose qu'on arrive à définir une catégorie ou des catégories gynomonoïques et la première étape est donc la caractérisation des individus. La figure 3 ci-contre montre la distribution des gynomonoïques selon leur pourcentage de fleurs femelles dans différentes populations. Il n'est pas possible de définir une catégorie discrète pour les gynomonoïques. La répartition est plutôt bimodale chez *S. nutans*, suggérant deux catégories d'individus, les hermaphrodites et les gynomonoïques à tendance hermaphrodite, les femelles et les gynomonoïques à tendance femelle. Chez *S. italica*, les gynomonoïques sont surtout proches des hermaphrodites.

En absence de données exploitables sur le déterminisme du phénotype sexuel, on s'est intéressé à la stabilité de ce dernier pour juger de l'importance de la part génétique, voir quels phénotypes pourraient se regrouper dans la même catégorie, essayer de mettre en évidence – ou non – un caractère adaptatif à ces phénotypes intermédiaires (voir paragraphe *2.4.2*).

Influence de la compétition entre graines et de la richesse du milieu sur le système de reproduction

Encadré 7

Compétition locale

Les graines (ou pour des animaux, descendants par la voie femelle) ont une dispersion limitée et les descendants d'un même individu se trouvent donc en compétition. La survie des plantules est dépendante de leur densité selon une fonction classique de compétition :

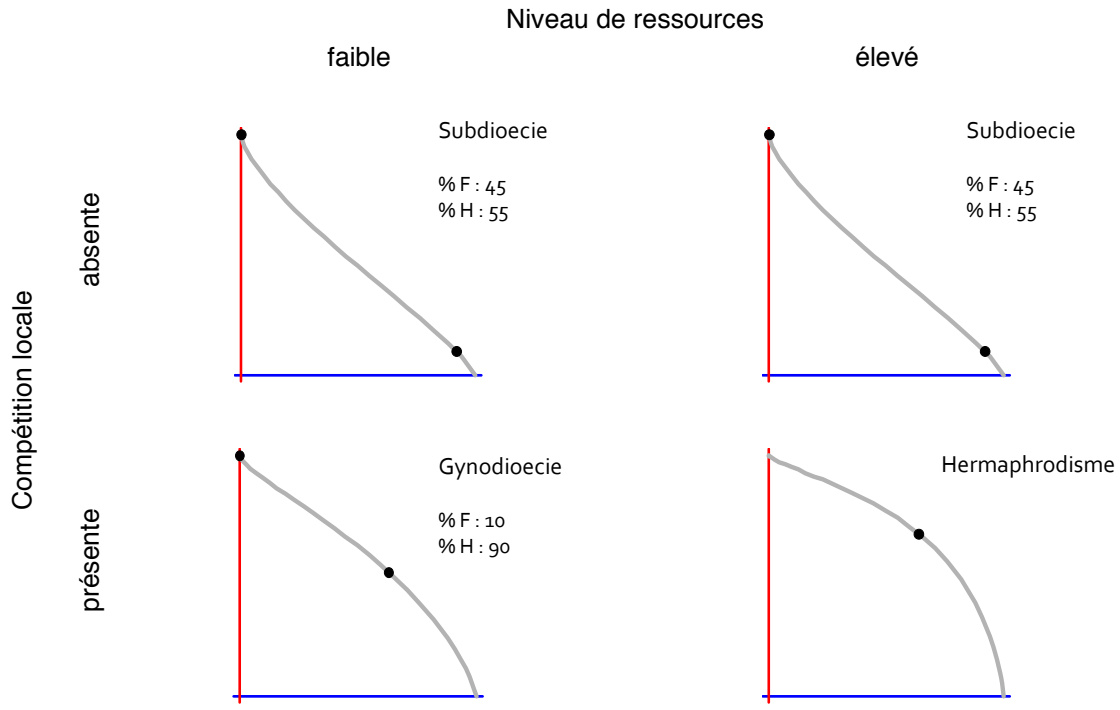
$$\text{Probabilité de survie d'une plantule} = \frac{s_0}{1 + a\left[\text{nombre de plantules dans la surface}\right]^b}$$

Avec s_0 probabilité de survie en absence de compétition, a et b paramètres décrivant l'intensité de la compétition, celle-ci augmente quand a et/ou b augmentent.

Influence sur le système de reproduction

Le modèle établi est phénotypique mais la méthode diffère de celle présentée dans l'encadré 3 en ce que les caractéristiques de l'hermaphrodite ne sont pas fixes mais sont déterminées conjointement au système de reproduction, selon la forme des courbes de gain des fonctions mâles et femelles (voir encadré 1) et les valeurs d'autres paramètres comme l'intensité de la compétition, le niveau de ressources, le taux d'autofécondation et la dépression de consanguinité.

La figure ci-dessous présente, pour un exemple de courbes de gain, la forme du fitness-set (gain mâle en abscisses, femelle en ordonnées), les types sexuels présents et leur fréquence selon des niveaux de ressources et de compétition, en absence d'autofécondation.



La compétition change l'allure du fitness-set puisqu'elle change le rendement de l'investissement dans la fonction femelle. La richesse du milieu n'a pas d'influence en absence de compétition, alors qu'elle change la forme du fitness-set et favorise les hermaphrodites en présence de compétition.

2.4 Trois phénotypes sexuels correspondant à une plasticité des individus - L'influence du milieu sur l'expression du sexe

Un changement de sexe de l'individu selon l'environnement peut être favorisé si les modalités de l'environnement ont une influence importante, différentielle selon le sexe, sur le succès reproducteur et si l'environnement est hétérogène et incontrôlable par le parent ou le descendant (Charnov & Bull, 1977). L'argumentation a surtout été développée pour les changements mâle/femelle mais peut être appliquée à l'allocation sexuelle des hermaphrodites. J'ai déjà signalé l'idée selon laquelle les milieux riches favoriseraient la fonction femelle. Une autre idée logique est que l'unisexualité serait favorisée dans les environnements pauvres. Cette idée est très répandue et supportée par quelques données : dans plusieurs espèces subdioïques, la forme pollinifère présente une fructification plus importante en environnement favorable (Delph & Wolf, 2005 pour une revue) et quelques cas sont connus de genres (resp. espèces) dans lesquels les espèces (resp. populations) dimorphiques sont trouvées dans des environnement difficiles alors que les espèces (resp. populations) monomorphiques sont trouvées dans des localités plus favorables, en général plus humides (Case & Barrett, 2001).

2.4.1 Le modèle Antirhea

Les données sur *Antirhea* ont montré que la fonction femelle des morphes pollinifères dépend du niveau de ressources du milieu (*2.3.1*). Une observation supplémentaire est que la quasi-totalité des plantules sont trouvées sous la canopée d'arbres adultes et que la compétition entre plantules est très forte. Ces deux phénomènes sont en interaction puisqu'une augmentation des ressources aura pour conséquence une augmentation de la production de graines d'un individu et donc de la compétition locale (entre apparentés) si la dispersion est insuffisante. Cette interaction a été étudiée de façon théorique avec Isabelle Litrico pendant sa thèse (2004).

Nos calculs montrent qu'une augmentation de la richesse du milieu ne change pas toujours le système de reproduction (encadré 7). Avec les formes classiques de fitness-set que nous avons considérées (gain dans une fonction = (investissement dans cette fonction)^k), la richesse n'a d'influence que lorsque la fonction femelle subit une compétition locale. Ce résultat est à rapprocher de l'étude de De Laguérie et al (1993) qui montrait que des courbes de gain sigmoïdes sont nécessaires pour que la richesse du milieu ait un effet sur le système de reproduction. Leur conclusion sur la forme des courbes de gain n'est bizarrement jamais reprise dans la littérature empirique.

Il faut signaler que ce modèle n'explique pas les données *Antirhea* puisque la compétition entre graines fait que ce sont les femelles qui doivent devenir hermaphrodites, pas les mâles. Il apparaît donc crucial de vérifier l'origine maternelle des graines ou plantules trouvées sous les arbres (analyse de maternité, pour changer) de façon à savoir si les fortes concentrations de plantules sous les canopées sont dues à un manque de dispersion ou à un effet perchoir. Dans ce dernier cas, la compétition n'est plus locale.

Figure 4 : Changements de catégorie phénotypique chez *Silene italica*, entre 2000 et 2001 (gauche, N=499), 2001 et 2002 (droite, N=303). Les données sont exprimées en pourcentage.

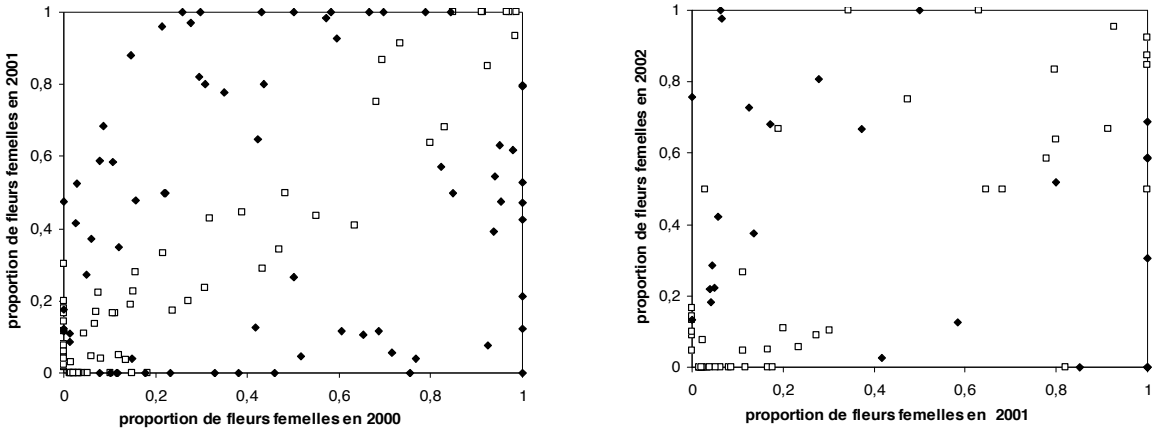


Figure 5 : **Variation inter annuelle de la proportion de fleurs femelles chez les individus variables de *S. italica*.** Les losanges noirs représentent les individus dont le changement de sexe est significatif lorsqu'on tient compte du nombre de fleurs produits (χ^2 ou test exact de Fisher).

Traitement	Nb de paires de clones		*P*	Nb moyen de fleurs par plante	
	ayant fleurit	ayant changé de sexe		contrôle	traitement
Substrat pauvre (sable)	24	4		46.0	9.8
Sécheresse	21	7	0.27	27.8	13.2
Pollinisation	27	11	0.62	25.3	16.9
Température 2001	40	16	0.17	25.6	14.0
Température 2002	41	17	0.016	45.1	30.6

Tableau 3 : Effet de différents traitements sur la proportion de fleurs femelles de clones de *S. italica*.

Je veux aussi préciser qu'une certaine confusion vient du fait qu'il faut bien distinguer les cas où le niveau de ressources disponibles pour la reproduction varie selon les individus au sein d'une même population, des cas dans lesquels on considère que les populations dans leur globalité se trouvent dans des milieux plus ou moins riches. Dans ce dernier cas, si les courbes de gain sont simples, linéaires, accélérantes ou saturantes, que les hermaphrodites s'autoféconden ou non, changer le niveau de ressource de la population ne change rien au système de reproduction stable, ni à l'allocation mâle/femelle des hermaphrodites. En revanche, si le niveau de ressources est variable entre individus de la même population, l'allocation mâle/femelle des hermaphrodites sélectionnée sera affectée par le niveau de ressources de l'individu même si les courbes de gain sont de forme simple (Klinkhamer *et al.*, 1997).

2.4.2 La gynodioecie-gynomonoecie

L'étanchéité des catégories femelle, gynomonoïque, hermaphrodite, et les facteurs pouvant influencer le pourcentage de fleurs femelles et éventuellement le passage d'une catégorie à l'autre ont été abordés de plusieurs manières.

La mesure du pourcentage de fleurs femelles des individus sur trois années consécutives montre que la majorité des échanges ont lieu entre les catégories hermaphrodite et gynomonoïque (figure 4). Néanmoins des échanges sont aussi observés avec les femelles de telle sorte que même cette catégorie ne peut être considérée comme complètement étanche, contrairement à ce qui est décrit dans les espèces subdioïques. Les changements de sexe (figure 5) sont tels qu'on ne peut pas non plus couper la population en une catégorie « plutôt hermaphrodite » et une « plutôt femelle ».

Il n'y a pas d'augmentation de la proportion de fleurs femelles avec l'âge (figure 5). Il n'y a aucune corrélation entre le changement de cette proportion et la taille de l'individu ou le changement de taille de l'individu.

Sur des clones ont été testés les effets des traitements « sable », « sécheresse » et « pollinisation » qui représentent des milieux à priori défavorables (l'idée pour le traitement « pollinisation » est que les fruits se développent et consomment des ressources). Ces facteurs du milieu qui pourraient se relier à des hypothèses adaptatives ne montrent aucun effet (tableau 3). Seule une augmentation de la température, dont on sait qu'elle se retrouve souvent dans les facteurs de changement de sexe (Korpelainen, 1998) mais qui n'a pas d'explication adaptative, augmente la proportion de fleurs femelles des clones.

Il faut bien avouer qu'à ce stade le statut des gynomonoïques n'est pas vraiment résolu et que je peux juste conclure que rien ne va dans le sens d'un caractère adaptatif de ce phénotype.

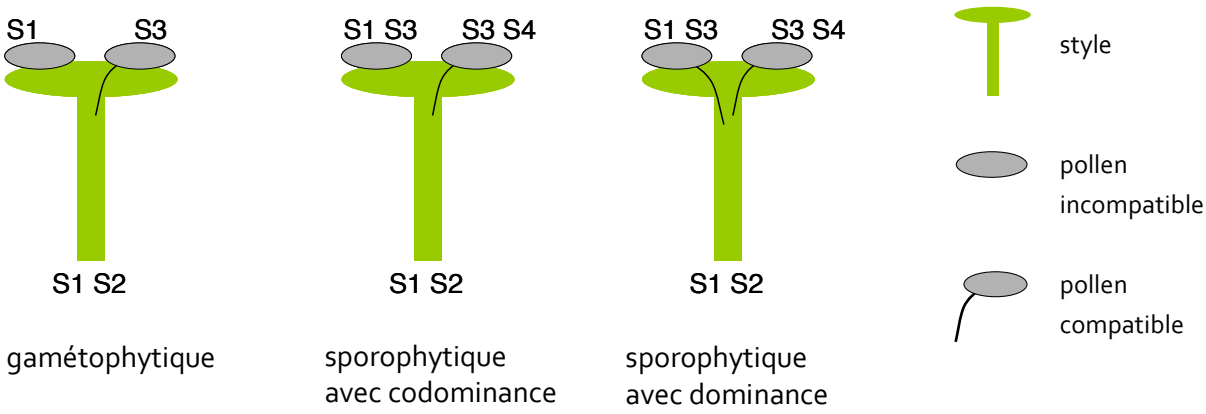


Figure 6 : Exemple de croisements compatibles ou incompatibles selon le type de système d'incompatibilité.

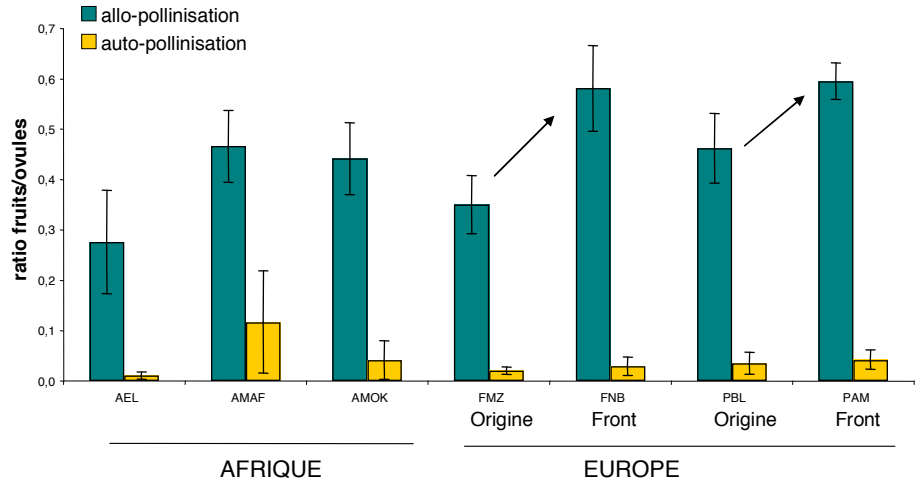


Figure 7 : Succès des allo- et auto-pollinisations pour différentes populations de *Senecio inaequidens*. L'Afrique est l'aire d'origine. Pour l'Europe, qui est l'aire envahie, deux transects indépendants de colonisations ont été étudiés. Seules les formes tétraploïdes ont envahi l'Europe (A14) et les populations africaines choisies pour la comparaison sont aussi tétraploïdes.

3. Quelques mots sur les systèmes d'incompatibilité homomorphiques

Chez les espèces possédant un système d'auto-incompatibilité homomorphiques, les individus – hermaphrodites –sont tous morphologiquement semblables mais diffèrent pour leur génotype à un super-locus gérant les possibilités de croisements. Deux individus seront compatibles s'ils possèdent des allèles différents à ce locus (appelé S-locus) : dans les systèmes gamétophytiques le génotype diploïde de la mère est confronté au génotype haploïde du grain de pollen, dans les systèmes sporophytiques le génotype diploïde de la mère est confronté au génotype diploïde du donneur de pollen (Figure 6). Les génotypes rares peuvent se croiser avec plus de partenaires, on a donc une sélection avec fréquence-dépendance négative et un polymorphisme important est maintenu S-locus.

Lors d'une colonisation on s'attend à des goulots d'étranglement qui ont la double conséquence de diminuer fortement, au moins momentanément, le nombre d'individus dans la population et de diminuer le nombre d'allèles d'incompatibilité présents et donc le pourcentage d'individus compatibles. Les systèmes d'incompatibilité sont donc défavorisés dans les espèces colonisatrices (Baker's law - Baker, 1967). Nous avons testé chez une espèce envahissante, *Senecio inaequidens* (Asteraceae - figure 1), si le niveau de compatibilité entre individus avait été réduit lors de la colonisation de la nouvelle aire et/ou si le système d'auto-incompatibilité avait été relâché (thèse de Lucile Lafuma, A19). Le peu de succès des autofécondations montre que l'espèce est restée fortement auto-incompatible lors de l'invasion (Figure 7) bien que le potentiel pour la sélection soit a priori présent puisque qu'une ou deux plantes par population montrent un taux d'auto-compatibilité assez fort. Le résultat le plus surprenant est l'augmentation du succès des allo-pollinisations le long des deux transects de colonisation étudiés (Figure 7). Cette augmentation est due à une augmentation du pourcentage de croisements compatibles entre l'origine et le front de chaque transect, ce qui est contraire aux prédictions puisqu'on s'attendait à un appauvrissement en allèles d'auto-incompatibilité au cours des colonisations successives.

Le fait que l'espèce soit restée fortement incompatible pourrait provenir du fait que les goulots d'étranglement se revèlent plus faibles que d'abord supposé. En effet, les revues récentes notent que les espèces envahissantes allogames montrent peu ou pas de diminution de variabilité génétique dans l'aire d'introduction (Novak & Mack, 2005 ; Bossdorf *et al.*, 2006).

L'augmentation du niveau d'allo-compatibilité le long des transects est plus difficile à expliquer. L'évolution de nouveaux allèles d'incompatibilité est très improbable à cette échelle de temps (Charlesworth, 2000). Une possibilité est liée au fait que les Asteraceae présentent un système sporophytique d'incompatibilité et que, dans ces systèmes, la dominance permet de cacher les allèles récessifs et d'augmenter le nombre de croisements compatibles (Figure 6). Une augmentation de l'allo-compatibilité pourrait donc se faire par un changement de fréquence des allèles selon leur niveau de dominance ou bien par un changement de niveau de dominance des allèles. Cette dernière possibilité a été invoquée par Brennan *et al* (2006) pour expliquer leurs résultats de croisement chez *Senecio squalidus*, une colonisatrice en Angleterre. Alternativement, l'augmentation de la compatibilité pourrait être due à une plus grande diversité présente aux fronts de colonisation si des mélanges d'origines variées ont eu lieu lors de la colonisation.

La fréquence-dépendance qui s'établit automatiquement entre les fonctions mâle et femelle permet de maintenir, sous certaines conditions, un polymorphisme stable de deux phénotypes. Pour qu'un troisième phénotype puisse être maintenu dans la même population, il faut une deuxième source de fréquence-dépendance : les mâles, femelles et hermaphrodites peuvent coexister de façon stable si par exemple leur fertilité est affectée par la limitation pollinique et que cette limitation dépend de la fréquence des producteurs de pollen dans la population ; ou si le déterminisme est nucléo-cytoplasmique et que les restaurateurs de la fertilité mâle ont un coût dépendant du contexte cytoplasmique. Lors d'une fréquence-dépendance à un niveau encore plus fin de phénotype, comme dans le cas de l'auto-incompatibilité, on maintient un grand nombre de phénotypes (théoriquement un nombre infini dans une population infinie). Plus le nombre de phénotypes, et donc de génotypes, est important, plus la taille et la dynamique des populations aura de conséquences. Ainsi dans le cas des déterminismes nucléo-cytoplasmiques, certains cas de trioecie dépendent des fréquences de départ et sont plus ou moins résistants à la dérive. Dans le cas de l'auto-incompatibilité, le nombre de génotype sera déterminé d'abord par la dérive et la taille de population pourrait avoir des conséquences sur les caractéristiques des allèles en plus de leur nombre. Cet aspect fait l'objet du projet 1 détaillé page suivante.

Le milieu peut avoir de l'influence sur le système de reproduction sélectionné. Ses effets escomptés sont souvent directement inclus dans la forme des courbes de gain ou du fitness-set sans être aucunement quantifiés. Par exemple une courbe femelle saturante pourra représenter un milieu où les disperseurs sont rares ou peu efficaces. Un facteur particulier du milieu peut aussi être plus précisément explicité pour être étudié en tant que tel comme on l'a montré pour la compétition et la richesse du milieu. Ces modèles montrent parfois qu'une modalité différente du facteur peut aboutir à un système de reproduction différent : ils prédisent donc que les phénotypes doivent changer avec le milieu mais ce ne sont en aucun cas des modèles d'évolution de la plasticité. De façon générale la littérature sur les systèmes de reproduction est déconnectée de la littérature sur l'évolution de la plasticité. Nous nous proposons dans le cadre de la thèse de Bojana Stojanova d'essayer de faire le lien entre les deux, par un commencement modeste sur un exemple particulier, c'est ce qui est détaillé dans le projet 2.

Projet 1 : **L'auto-incompatibilité chez la plante envahissante *Senecio inaequidens* (Asteraceae): un cas d'évolution de la dominance ?**

Le contexte

Des systèmes génétiques d'auto-incompatibilité sont présents dans de nombreuses familles de plantes et jouent un rôle essentiel dans le régime de reproduction de ces espèces en favorisant la reproduction entre non-apparentés. Ces systèmes posent un problème particulier en biologie de la conservation: une petite taille de population mène à une réduction du nombre d'allèles d'auto-incompatibilité, ce faible nombre cause lui-même une réduction du nombre de partenaires possibles et donc éventuellement une diminution de la fertilité des individus.
Les systèmes d'auto-incompatibilité subissent une sélection fréquence-dépendante puisque les génotypes les plus rares sont compatibles avec plus de partenaires et se reproduisent mieux. Ces systèmes présentent donc un grand polymorphisme et une grande hétérozygotie (théorique et réalisée).

Les Asteraceae possèdent un système d'incompatibilité sporophytique. Dans de tels systèmes le mécanisme de reconnaissance dépend des génotypes diploïdes du père et de la mère et les allèles peuvent être codominants entre eux ou montrer des relations de dominance. Lorsqu'il y a dominance, certains allèles ne s'expriment pas et les croisements sont plus souvent compatibles (ex : les génotypes S1S2 et S1S3 sont incompatibles si les allèles sont codominants, ils sont compatibles si un des allèles, S2 ou S3, est dominant sur S1). Des développements théoriques récents ont montré que :

- Les allèles les plus dominants étant plus visibles à la sélection fréquence dépendante, ils résistent mieux à la dérive que les allèles récessifs. La fréquence des classes de dominance et le nombre d'allèles par classe sont donc affectés par la taille des populations (Billiard *et al.*, 2007).
- Les propriétés de dominance des allèles peuvent elles-même évoluer : une évolution de la dominance au niveau de l'expression dans le pollen est facilitée par la présence d'un faible nombre d'allèles d'incompatibilité dans la population ; une évolution de la dominance de l'expression dans le style est également possible mais seulement si la fertilité femelle est limitée par le nombre de partenaires compatibles (Schoen & Bush, 2009).

Les populations invasives, qui ont subi des goulots d'étranglement, remplissent donc a priori les conditions favorisant les changements de fréquences des classes de dominance (petite taille des populations) et les conditions favorisant l'évolution de la dominance (peu d'allèles présents, éventuellement limitation de la fertilité femelle des individus). D'autre part les modèles théoriques généraux d'évolution de la dominance montrent que celle-ci nécessite un fort taux d'hétérozygotes puisque c'est le compartiment où s'exerce la sélection (Otto & Bourguet, 1999), le système d'incompatibilité semble donc être idéal pour tester l'évolution de la dominance *in natura*.

Le projet

Notre modèle d'étude est le complexe d'espèces *Senecio inaequidens*-S. *madagascariensi*s (Asteraceae). Ce complexe, natif d'Afrique du Sud, présente des populations diploïdes et tétraploïdes (A14). Les formes tétraploïdes envahissent l'Europe (sous le nom de *S. inaequidens*) tandis que les formes diploïdes envahissent Hawaï et l'Australie (sous le nom de *S. madagascariensis*). Les individus sud-africains sont auto-incompatibles et les premières études (A19) ont montré *(i)* que les plantes européennes sont restées fortement auto-incompatibles et *(ii)* que le taux de compatibilité entre plantes a augmenté le long des fronts d'invasion (deux transects d'invasion a priori indépendants, en France et Belgique - Pays-Bas, ont été étudiés). Ces résultats sont contraires aux prédictions puisqu'on s'attend à une diminution du nombre d'allèles d'auto-incompatibilité lors de la succession de goulots d'étranglement et donc à une diminution du taux de partenaires compatibles. Deux types d'explications sont envisageables : 1) pendant longtemps *S. inaequidens*

est resté cantonné près des sites historiques d'introduction avant de s'étendre en Europe, s'il y a eu des mélanges de populations d'origine différentes ('*admixture*') lors de cette extension, la diversité neutre sera plus grande dans les populations le long du front que dans les populations « historiques », et la diversité des allèles d'incompatibilité également ; 2) il y a eu changements des fréquences d'allèles selon leur classe de dominance, ou une évolution des propriétés de dominance même des allèles (comme expliqué ci-desssus).

Afin de tester les différentes hypothèses, nous proposons donc de:

1- Caractériser la diversité génétique neutre dans les populations européennes et en particulier dans les populations utilisées dans les croisements afin de tester l'hypothèse d'*admixture*. Une phylogéographie vient de paraître (Lachmuth *et al*., 2010) qui retrace les routes d'invasion et confirme que les colonisations française et belge sont d'origine différente. Cette étude montre une diminution de la diversité sur le transect belge-néerlandais, et ne comprend pas le transect français.

2- Effectuer des plans de croisements plus complets pour des populations européennes choisies. Pour connaître le nombre de classes de dominance et estimer le nombre d'allèles dans chaque classe, il faut effectuer des croisements en plan di-allelique complet pour chaque population. On compare ensuite les populations entre elles. Pour repérer des changements de propriété de dominance des allèles, il faut en plus effectuer des croisements entre populations afin d'établir les correspondances entre les catégories trouvées dans chaque population.

3- Etendre cette étude aux individus diploïdes qui envahissent l'Australie. D'une part, les croisements sur des diploïdes seront plus faciles à interpréter et d'autre part, l'effet de la dominance étant plus fort chez les tétraploïdes (dissimulant plus d'allèles), les diploïdes et les tétraploïdes n'ont pas forcément évolué de la même manière.

L'aspect le plus novateur et fondamental de l'étude est l'évolution de la dominance mais cette étude amènera aussi des jalons dans les domaines plus pratiquement liés au maintien et à la dynamique de la Biodiversité: (*i*) comprendre l'évolution et la dynamique de l'auto-incompatibilité dans un contexte d'invasion biologique (*ie*. populations encore en expansion et avec des densités différentes selon leur position par rapport au front d'invasion); et (*ii*) mieux appréhender dans le futur la propagation de *S. inaequidens* – très préoccupant en Europe - en quantifiant les conséquences que peuvent avoir des mélanges de populations sur la biologie de la reproduction et par conséquent sur la dynamique des populations.

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Projet 2 : *Evolution de la plasticité : le cas des systèmes de reproduction.*

Le contexte

La plasticité

Les individus ont plusieurs moyens de faire face à un environnement changeant : se déplacer dans l'espace pour suivre les conditions favorables, modifier leur environnement quand cela est possible, s'adapter aux nouvelles conditions par déplacement de caractère ou par plasticité. La plasticité phénotypique est la capacité d'un génotype à produire des phénotypes différents en fonction de l'environnement expérimenté par ce génotype. La plasticité phénotypique est adaptative lorsque le changement de phénotype permet d'améliorer la valeur sélective de l'individu dans le nouvel environnement. L'ensemble des phénotypes que peut produire un génotype dans les différents environnements est appelé norme de réaction.

La plasticité sera sélectionnée si elle a une base génétique et si les individus plastiques ont en moyenne une meilleure valeur sélective que les individus non plastiques. Ce dernier point nécessite que :

- l'environnement soit variable ;
- les conditions environnementales soient, en partie au moins, prévisibles ;
- le gain apporté par le changement de phénotype soit supérieur aux coûts de la plasticité.

Les coûts de la plasticité sont ceux liés à la maintenance des fonctions « sensorielles » - nécessaires à l'appréhension de l'environnement - et des fonctions « régulatrices » – responsables des changements phénotypiques. Un autre type de coût peut être lié à la production du phénotype si les structures sont plus coûteuses quand elles sont réalisées de façon plastique plutôt que de façon fixe. Les limites aux bénéfices de la plasticité peuvent provenir de la difficulté à appréhender correctement, et à temps, l'environnement et du fait que des génotypes plastiques peuvent être moins performants que des génotypes « fixes » dans la production des phénotypes extrêmes (De Witt *et al.* 1998).

Particularité des systèmes de reproduction quant à l'évolution de la plasticité

La valeur reproductive d'un phénotype (ici une stratégie sexuelle) peut dépendre de l'environnement. Une stratégie hermaphrodite permettra une meilleure production de gamètes en milieu riche alors qu'une stratégie unisexuée sera favorisée en milieu pauvre, par exemple. Ou encore, un milieu pauvre en pollinisateurs favorisera une stratégie autogame pouvant se passer d'agent pour sa reproduction. En ce sens une détermination du phénotype sexuel en partie plastique pourrait être sélectionnée.

Mais les systèmes de reproduction présentent une particularité forte, qui est que la valeur d'un phénotype est dans la plupart des cas fréquence-dépendante. On le comprend aisément dans le cas des polymorphismes sexuels sur les fonctions mâle et femelle : le succès reproducteur d'un mâle dépendra de la fréquence relative des mâles et des femelles dans la population. De façon moins évidente le régime de reproduction, défini comme la part de reproduction effectuée en allo ou en auto-fécondation, est aussi soumis à une certaine fréquence dépendance. Pour chaque descendant produit, les individus pratiquant l'autogamie laissent une copie supplémentaire de leurs gènes, par rapport aux individus allogames, puisqu'ils fécondent eux-mêmes leurs gamètes femelles en plus de féconder ceux des autres individus. Ce phénomène est appelé l'avantage automatique à l'autofécondation. Cet avantage peut être contrebalancé par la dépression de consanguinité qui rend les individus issus d'autofécondation moins performants. L'importance de cet avantage dépend aussi du « pollen discounting » qui désigne la proportion de pollen qui a été utilisé pour l'autofécondation, diminuant ainsi le pollen exporté pour l'allofécondation et de la

fréquence des gamètes disponibles pour l'allofécondation - c'est là qu'intervient la fréquence des différents types.

Le fait que le succès d'un phénotype sexuel dépende de la fréquence des différents phénotypes dans la population implique qu'il n'y aura pas toujours de phénotype optimum pour un milieu donné. Les conditions d'évolution de la plasticité – et les méthodes pour étudier cette évolution – seront donc différentes des cas classiques dans lesquels on considère que le phénotype A est le meilleur dans l'environnement 1 et le phénotype B le meilleur dans l'environnement 2. Les modèles d'optimisation ne sont plus fonctionnels dans notre cadre.

Le projet

Les développements théoriques

Nous désirons développer des modèles pour quantifier l'importance de la sélection fréquence-dépendante dans l'évolution de la plasticité, ou plus précisément dans la limitation de cette évolution puisque l'idée intuitive est que la fréquence-dépendance limitera les avantages de la plasticité.

Le caractère d'intérêt est la plasticité mais la valeur sélective des individus dépend d'un trait sous-jacent (par exemple le taux d'autofécondation). La formalisation de l'évolution de la plasticité n'est pas triviale puisque pour une norme de réaction donnée la valeur moyenne du trait dépendra de la fréquence des différents environnements. Une difficulté que nous devrons résoudre est donc séparer la sélection sur la variance du trait (c'est-à-dire la plasticité elle-même) de la sélection sur la moyenne du trait.

Pour les régimes mixtes (mélange d'autofécondation et d'allofécondation) nous avons expliqué ci-dessus que la fréquence-dépendance provient du fait que le succès du pollen à l'exportation dépend de la proportion d'ovules accessibles L'importance de cette fréquence-dépendance doit logiquement diminuer si la quantité de pollen exporté par les fleurs autofécondées diminue (« pollen discounting »). Une première idée est donc d'utiliser le taux de « pollen discouting » pour faire varier l'importance de la fréquence-dépendance.

Les études expérimentales
Le modèle biologique utilisé est le *Lamium amplexicaule* (Lamiaceae) qui est une espèce dite cléistogame : elle présente la particularité de produire sur un même pied des fleurs fermées (cléistogames) qui s'autofécondent forcément, et des fleurs ouvertes (chasmogames) qui sont potentiellement allofécondées. Ce mode de reproduction a évolué indépendamment dans une cinquantaine de familles d'Angiospermes (Culley & Klooster 2007).

Dans les espèces non cléistogames, on peut éventuellement observer une variation du taux d'autofécondation en fonctions des conditions environnementales mais on ne peut savoir s'il s'agit d'une conséquence directe (par exemple : un temps trop froid peut mener à une absence de pollinisateur, donc à une absence d'allofécondation, ce qui augmente automatiquement le taux d'autofécondation) ou s'il y a eu une réponse plastique adaptative de la plante. L'avantage des espèces cléistogames est que l'on peut observer un taux de fleurs ouvertes ou fermées qui définissent a priori la stratégie de la plante. De plus plusieurs études ont montré que certaines espèces cléistogames sont capables de réguler la proportion de fleurs ouvertes produites en fonction de signaux environnementaux comme la photopériode et la température (Lord 1982 ; Winn & Moriuchi 2009). Elles montrent donc une vraie plasticité, et si ce phénomène de plasticité du système reproducteur permet à la plante d'avoir une meilleure valeur sélective en moyenne sur les environnements rencontrés, alors il s'agit de plasticité adaptative.

Le *Lamium amplexicaule* fleurit une fois par an – au printemps - dans le sud de la France et fleurit deux fois par an - au printemps et en automne - à des latitudes plus élevées. En Bourgogne des

observations préliminaires montrent que le taux de fleurs fermées est plus important en automne. L'idée est que ce comportement plastique peut être expliqué par l'adaptation de la plante à la présence/absence des pollinisateurs dans les différentes conditions de floraison.
1. Nous allons donc utiliser les différentes origines (Languedoc et Bourgogne) pour tester l'hypothèse selon laquelle les plantes soumises habituellement à deux types d'environnements contrastés sont plus plastiques que celles qui ne fleurissent qu'au printemps.
2. La mesure du succès des fleurs fermées et des fleurs ouvertes, au printemps et à l'automne, nous permettra de calculer le phénotype optimum pour chaque saison, et donc le phénotype plastique idéal. La comparaison entre ce phénotype idéal et les phénotypes effectivement observés nous renseignera sur les limites (ajustement correct aux facteurs environnementaux) et les coûts (nombre de fleurs produites et succès moyen par fleur des individus très plastiques / aux individus fixes ou moins plastiques) de la plasticité dans notre système. Ces limites et coûts sont des paramètres des modèles.
3. La mesure du succès reproducteur et de la « favorabilité » des saisons nécessite la connaissance du taux d'autofécondation des fleurs ouvertes au printemps et en automne (estimation par marqueurs moléculaires), ainsi que la connaissance de la dépression de consanguinité. Ces deux paramètres seront également mesurés.

Ce modèle biologique sera confronté au travail théorique mais le travail théorique est de portée beaucoup plus large puisqu'il concernera tous les systèmes de reproduction mixtes. On peut de plus espérer que les méthodologies développées pourront servir à étudier de façon générale l'importance de la fréquence-dépendance dans l'évolution de la plasticité.

Ce projet se situe dans le cadre de la thèse de Bojana Stojanova qui est sous la co-direction de Pierre-Olivier CHEPTOU du CEFE et moi-même.

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Evolution of dioecy: can nuclear–cytoplasmic interactions select for maleness?

SANDRINE MAURICE*, ERIC BELHASSEN†, DENIS COUVET‡ & PIERRE-HENRI GOUYON

Laboratoire d'Evolution et Systématique des Végétaux, Bat 362, Université Paris Sud, 91 405 Orsay Cedex, †ENSAM, 2 Place Pierre Viala, 34000 Montpellier and ‡Laboratoire d'Evolution, CEFE, Route de Mende, BP 5051, 34033 Montpellier Cedex, France

A model of evolution of reproductive systems, when sex is determined by both nuclear and cytoplasmic genes, is presented. Such a control of sex is known to facilitate the occurrence of female individuals in hermaphroditic populations, thus leading to gynodioecy. A two-cytotypes two-nuclear loci (two alleles at each nuclear locus) model for gynodioecy has been developed previously. Such gynodioecious systems are usually considered as stable, i.e. not leading to dioecy. In order to find out if the presence of females can select for male individuals when sex determination is nuclear–cytoplasmic, we followed the evolution of alleles responsible for female sterility. These alleles can be at the preceding loci or at a third locus. We show that male individuals can be selected. Dioecy evolves in less restrictive conditions than under nuclear sex determination. The same also holds for trioecy (coexistence of females, hermaphrodites and males). Nuclear–cytoplasmic polymorphism can be maintained in these reproductive systems.

Keywords: cytoplasmic male sterility, dioecy, gynodioecy, reproductive systems, sex determination, trioecy.

Introduction

The evolution of dioecy from the hermaphrodite state has long been discussed but is still not fully understood. The selective force most commonly put forward is the avoidance of inbreeding (see Thomson & Barrett, 1981, for references). Other explanations are based on resource allocation theories. Different sexual forms can be more or less efficient in gamete production, depending on their biology (Charnov *et al.*, 1976). The total number of gametes produced by an individual can influence the chance of success of each gamete depending on the mode of pollination and seed dispersal; the evolution of dioecy has thus often been thought to be related to ecological conditions (Willson, 1979; see Thomson & Brunet, 1990, for a review and discussion). All these explanations deal with the number of gametes that the different sexual types pass to the next generation and are synthesized by Charnov's 'evolutionary stable strategy' calculations based on a phenotypic model of sexual strategies (Charnov *et al.*, 1976). Numerous genetic models involving male-sterile and female-sterile nuclear mutations also exist. These models give the same results as Charnov's calculations except when they deal with genetic constraints or with inbreeding (Charlesworth & Charlesworth, 1978; Gregorius *et al.*, 1983).

Charlesworth & Charlesworth (1978) have studied the influence of the recombination rate between the male sterility and the female sterility loci on the conditions that lead to dioecy, with different dominance coefficients of the sterility alleles: such constraints lead to equal or more restricted conditions for the evolution of dioecy compared with phenotypic models. Another genetic phenomenon that is known to influence the outcome of selection on sexual phenotypes is nuclear–cytoplasmic sex determination. Such a determination has been found in many wild and cultivated species (see Kaul, 1988, for a review). There are three reasons why the nuclear–cytoplasmic determination of sex can be important.

1 We already know that cytoplasmic or nuclear–cytoplasmic determination of sex allows the maintenance of gynodioecy under conditions when the population would be hermaphroditic if sex were controlled only by nuclear genes. These conditions are given by the relative female fertility of the two sex morphs, females and hermaphrodites. Several models show that cytoplasmic polymorphism, and thus females, can be maintained (Lewis, 1941; Lloyd, 1974: pollination limitation in females; Charlesworth, 1981;

*Correspondence.

Delannay *et al.*, 1981; Ross & Gregorius, 1985; Gouyon *et al.*, 1991: deleterious effect of the nuclear allele restoring male function; Frank, 1989: mutation and drift affecting cytotypes and nuclear restorers together with a deleterious effect of restorers).

2 Results from these models, combined with observations of high frequencies of females in natural populations of gynodioecious species with nuclear–cytoplasmic determination of sex (*Plantago lanceolata*: Baker, 1963; Van Damme, 1983; *Thymus vulgaris*: Dommee *et al.*, 1978; Belhassen *et al.*, 1991; *Origanum vulgare*: Kheyr-Pour, 1980) raise the question of how the selection on the sex allocation of individuals producing pollen is affected. Indeed, the higher the frequency of females, the higher should be the success of male gametes. Selection for a male-biased sex allocation in hermaphrodites or even for completely male individuals is thus expected. This has been shown to be true for nuclear control of male sterility (Charlesworth & Charlesworth, 1978).

3 Gynodioecy and dioecy are associated at the family level (88 per cent of families with gynodioecious species also contain dioecious species, a proportion that is much higher than in the whole flora: Maurice *et al.*, 1993) and gynodioecy often involves nuclear–cytoplasmic determination of sex. We thus wonder if nuclear–cytoplasmic gynodioecy can lead to dioecy?

The question of male allocation in hermaphrodites has been explored when there is an entirely male-sterile cytotype (all individuals having this cytotype are female; cytotype here designates the cytoplasmic genotype, not the level of ploidy) and a second fully male-fertile one (all individuals having this cytotype are hermaphroditic). In this case, the fertile cytotype acts as an isolated compartment for genes involved in pollen production (such as female-sterile alleles), so that conditions for males to evolve are the same as with nuclear sex determination (Charlesworth, 1989; Maurice *et al.*, 1993; see Werren, 1987, for a similar process in the evolution of sex ratio). When one cytotype is fertile and one contains females and hermaphrodites, hermaphrodites can sometimes be selected to invest more in male function, but the fertile cytotype is then quickly lost (Maurice *et al.*, 1993). Genetic studies (*Plantago lanceolata*: Van Damme, 1983; *Thymus vulgaris*: Belhassen *et al.*, 1991) have shown that several cytotypes causing male sterility can be found within a single population. The presence of females in several cytotypes can be crucial for the maintenance of cytoplasmic polymorphism. Here we consider an extension of the simplified sex detemination proposed by Van Damme (1985) for gynodioecy in *Plantago*: there are two potentially male-sterile cytotypes and a nuclear locus that restores male fertility for each cytotype. This model has been explored for gynodioecy by Gouyon *et al.* (1991). In the following model, both cytotypes can yield females, hermaphrodites and males, depending on the nuclear genes. Using computer calculations, we search for the conditions that allow males to invade a gynodioecious population with such a determination of sex and compare these conditions with those obtained when the determination of sex is nuclear.

Model

The model is similar to the one described in Gouyon *et al.* (1991) which was based on the situation encountered in *Plantago lanceolata* (Van Damme, 1983). There are two cytotypes. Corresponding to each cytotype (*C1, C2*), there exist nuclear loci (*1, 2*) which control restoration of male fertility (i.e. pollen production). Being male or hermaphrodite can be controlled by different restorer alleles or by modifiers of sex allocation at other loci. We thus have considered two possible models for the control of sex. In the two-locus model (Table 1), both loci (*1, 2*) have three alleles: one allele that does not restore male fertility (usually called maintainer alleles: *r1, r2*) and two alleles that restore male fertility. One restorer allele makes an individual hermaphroditic (*R1, R2*), the other makes complete males (*R'1, R'2*). In the three-locus model (Table 2), each restorer locus (*1, 2*) has a maintainer allele (*r1, r2*) and a restorer allele (*R1, R2*). Individuals restored for male fertility are hermaphrodite or male depending on their genotype at a third nuclear locus (*M/m*). We assume that the restorer alleles are dominant over the maintainer or female alleles, which is the most commonly observed case (Kaul, 1988, p.121). Most calculations were made with the male alleles dominant over the hermaphroditic alleles, but we also calculated the reverse to check if it influences the results. In both models the loci are assumed to be unlinked. The notation is the same as in Charnov *et al.* (1976): β is the relative female fertility of hermaphrodites compared with females and α is the relative male fertility of hermaphrodites compared with males. Following the standard resource allocation theory, unisexual individuals are better in their specific function than hermaphrodites. The parameters α and β thus vary between zero and one. Restorer alleles are assumed to have a pleiotropic deleterious effect (Charlesworth, 1981; Delannay *et al.*, 1981; Frank, 1989). This deleterious effect, or cost, is denoted by d and is the diminution in fertility of individuals bearing a restorer allele compared with individuals bearing only the maintainer allele at the corresponding locus. This cost can affect

Table 1 Sexual phenotype and fertilities of individuals of cytotype *C1* depending on their genotype, for the two-locus model of sex determination. A similar table can be written down for individuals of cytotype *C2*. $d_m i$, $d_m' i$, $d_f i$ and $d_f' i$ denote the pleiotropic negative effects on male and female fertilities of the hermaphrodite and male restorer alleles of cytotype *i*. The male restorer alleles (*R'1* and *R'2*) are dominant over the hermaphrodite restorer alleles (*R1* and *R2*). The genotype at locus 2 does not affect the sexual phenotype of *C1* individuals but does affect their fertilities. Individuals *CiR'i*.. are male. Therefore, ovules *CiR'i*. and genotypes *CiR'iR'i*.. cannot exist

		Genotype at locus 2					
		r2r2		*R2r2* or *R2R2*		*R'2r2* or *R'2R2* or *R'2R'2*	
Genotype at locus 1	Sexual phenotype	Male fertility	Female fertility	Male fertility	Female fertility	Male fertility	Female fertility
r1r1	F	0	1	0	$1-d_f 2$	0	$1-d_f' 2$
R1r1 or *R1R1*	H	$\alpha(1-d_m 1)$	$\beta(1-d_f 1)$	$\alpha(1-d_m 1)(1-d_m 2)$	$\beta(1-d_f 1)(1-d_f 2)$	$\alpha(1-d_m 1)(1-d_m' 2)$	$\beta(1-d_f 1)(1-d_f' 2)$
R'1r1 or *R'1R1*	M	$1-d_m' 1$	0	$(1-d_m' 1)(1-d_m 2)$	0	$(1-d_m' 1)(1-d_m' 2)$	0

female fertility (d_f) or male fertility (d_m). In the two-locus model, the hermaphrodite restorer allele and the male restorer allele need not have the same cost (Table 1). In the three-locus model, only restorer alleles have cost (loci *1* and *2*) and the male allele at the third locus has no effect on females. The fertilities of the different genotypes are given in Tables 1 and 2.

The cytotype is assumed to be exclusively maternally transmitted. Hermaphrodites are assumed not to self-fertilize, so that gametes combine at random. The dominance assumed for the sexual phenotypic effect of alleles is also applied to their deleterious effects (see Tables 1 and 2). We present the results obtained when the parameter values are the same for both cytotypes and when the male and hermaphrodite restorer alleles have the same cost, so that the differences between individuals are restricted to their sexual phenotype. The model thus has four parameters: α, β, d_m and d_f, which are constant for each calculation.

The cost of the restorer alleles was arbitrarily set at 5 per cent (low cost) and 20 per cent (high cost), on one or both fertilities. The recursions describing change in genotype frequencies are given in the Appendix. Starting with arbitrary frequencies of female and hermaphrodite genotypes, we first let the population evolve until an equilibrium was reached to find the range of β values for which nuclear–cytoplasmic gynodioecy is obtained. The equilibrium was defined by a change in genotype frequencies of less than 10^{-4} over 100 generations. Values of β differing by steps of 0.01 were used to find the limit for gynodioecy. Gynodioecious populations contain both cytoplasmic types (*C1, C2*) and *riri, riRi, RiRi* genotypes at both restorer loci. Then, for different values of β, males were introduced at low frequency after the gynodioecious equilibrium was reached. For the two-locus model the genotypes *C1R'1R1R2r2* and *C2R1r1R'2R2* were introduced at a frequency of 0.005 each. We also carried out calculations introducing first the male restorer active in one cytotype (*R'1*) and introducing the second male restorer (*R'2*) only after the new equilibrium was reached. For the three-locus model, the genotype *C1R1r1R2r2Mm* was introduced at a frequency of 0.005. Using a step of 0.01 for α, we found the range of α for which males increase in frequency when rare ($\alpha < \alpha 1$) and the range of α for which males increase in frequency and completely displace the hermaphrodites ($\alpha < \alpha 2$). Again, the final state corresponds to a change in genotype frequencies of less than 10^{-4} over 100 generations. We verified that the final state was a stable equilibrium by carring out the reverse calculations, i.e. starting with a dioecious population and introducing hermaphrodites at low frequency. The final state was usually approached

Table 2 Sexual phenotype and fertilities of individuals of cytotype *C1* depending on their genotype for the three-locus model of sex determination. A similar table can be written down for individuals of cytotype *C2*. Notations are the same as in Table 1. The male allele (*M*) is dominant over the hermaphrodite allele (*m*). The genotype at locus 2 does not affect the sexual phenotype of *C1* individuals but does affect their fertilities

			Genotype at locus 2			
			r2r2		*R2r2* or *R2R2*	
Genotype at locus 1	Genotype at locus 3	Sexual phenotype	Male fertility	Female fertility	Male fertility	Female fertility
r1r1	*mm*	F	0	1	0	$1-d_f2$
	Mm or *MM*	F	0	1	0	$1-d_f2$
R1r1 or *R1R1*	*mm*	H	$\alpha(1-d_m1)$	$\beta(1-d_f1)$	$\alpha(1-d_m1)(1-d_m2)$	$\beta(1-d_f1)(1-d_f2)$
	Mm or *MM*	M	$1-d_m1$	0	$(1-d_m1)(1-d_m2)$	0

Table 3 Frequency of females in gynodioecious populations, depending on the relative female fertility of hermaphrodites compared with females (β), when the control of sex is nuclear or nuclear–cytoplasmic. When the control of sex is nuclear–cytoplasmic, the frequency of the restorer allele *Ri* at locus i ($i=1$ or 2) is indicated in parentheses. The frequency of each cytotype is 0.5

	Control of sex				
		Nuclear–cytoplasmic			
β	Nuclear	$d_m=0.05$ $d_f=0$	$d_m=0.05$ $d_f=0.05$	$d_m=0.20$ $d_f=0$	$d_m=0.20$ $d_f=0.20$
0.2	0.375	0.383† (0.452)	0.400 (0.436)	0.415 (0.426)	0.489 (0.358)
0.4	0.167	0.179 (0.620)	0.218 (0.578)	0.228 (0.570)	0.378‡ (0.432)
0.5	0	0.021 (0.862)	0.082 (0.736)	0.090 (0.722)	0.306 (0.486)
0.6	—	—	—	—	0.219 (0.560)
0.8	—	—	—	—	0.020 (0.862)

A — means that females are not maintained under these conditions.
† and ‡ The two cases discussed in the text.

rapidly (less than 100 generations) except near the limit values of α.

We verified that the program gave the same results as analytical calculations for nuclear determination of sex, when all the genotypes of one cytotype were set to zero at the beginning of the calculations.

Results

Nuclear–cytoplasmic polymorphism

As already explained in previous papers (Charlesworth, 1981; Delannay *et al.*, 1981; Gouyon *et al.*, 1991), the cost of restorer alleles allows for the maintenance of joint polymorphism, i.e. polymorphism for the cytotypes and for the restorer loci, in gynodioecious reproductive systems. We found here that this also holds for other reproductive systems whenever the maintainer alleles are not lost, i.e. when the females are not eliminated. The joint polymorphism is thus maintained under gynodioecy, dioecy and trioecy (coexistence of females, males and hermaphrodites). The mechanism responsible for the maintenance of this polymorphism is the same as the one detailed in Gouyon *et al.* (1991) for gynodioecy.

Conditions for nuclear–cytoplasmic gynodioecy

Females are maintained or not depending on the relative fertility of hermaphrodites compared with females (β) and the cost of restoration. As β increases, the frequency of females decreases. The calculations are deterministic (without drift) and a very low frequency of females allows the maintenance of joint polymorphism. The value of β above which the female frequency is less than 0.1 per cent is considered as the limit for gynodioecy. When the cost is 5 per cent and on male function only, the limiting β is 0.51; when it is on both fertilities, the threshold β is 0.55. When the cost is 20 per cent and on male function only, the threshold β is 0.57; when it is on both fertilities, it is 0.82. The limit of β for the maintenance of females under nuclear sex determination is 0.5 (Lewis, 1941). Examples of frequencies of females in gynodioecious populations with a nuclear or a nuclear–cytoplasmic determination of sex are given in Table 3.

Conditions for the evolution of dioecy

For some parameter values males increase in frequency but do not eliminate the hermaphrodites. The rate of increase of the male alleles at the beginning of the process depends on whether the male alleles are dominant over the hermaphrodite alleles, or the reverse, but the final result is never different. The threshold of the male fertility of hermaphrodites compared with males under which a rare male phenotype increases in frequency is denoted by $\alpha 1$. The value for which males increase in frequency and completely eliminate the hermaphrodites is denoted by $\alpha 2$. These values are given in Table 4 for the two genetic models for maleness. In every case, the values ($\alpha 1$ and $\alpha 2$) are equal to or higher than the corresponding values for a nuclear sex determination. This means that males both increase and invade under less restrictive conditions with nuclear–cytoplasmic control of sex. The difference in conditions is, however, usually quite small. It becomes larger as the cost of restorer alleles increases and/or affects both fertilities. The comparison of Tables 3 and 4 (for example cases † and ‡) shows that the change on the threshold α is linked to the increase in female frequency compared with nuclear control of sex. It does not depend on whether the cytotypes are more or less restored (the frequencies of the restorer alleles are similar in the two cases).

The joint polymorphism is maintained under dioecy, i.e. both *C1* and *C2* as well as the polymorphism at the nuclear restorer loci are maintained. In such dioecious systems, the frequency of females always exceeds 50

Table 4 Limit on the relative male fertility of hermaphrodites compared with males (α) for the increase of males in the population: if $\alpha \leq \alpha 1$ then males, if rare, increase in the population; if $\alpha \leq \alpha 2$ then hermaphrodites are eliminated from the population, i.e. $\alpha 2$ is the threshold for dioecy. If $\alpha 2 < \alpha \leq \alpha 1$ males and hermaphrodites coexist, i.e. the reproductive system is trioecy (except in the two nuclear cases denoted by § in which females are not present). For each set of parameters, the first line gives the results for the two-locus model and the second line the results for the three-locus model

	Control of sex									
			Nuclear–cytoplasmic							
	Nuclear		$d_m = 0.05$ $d_f = 0$		$d_m = 0.05$ $d_f = 0.05$		$d_m = 0.20$ $d_f = 0$		$d_m = 0.20$ $d_f = 0.20$	
β	$\alpha 1$	$\alpha 2$	$\alpha 1$	$\alpha 2$	$\alpha 1$	$\alpha 2$	$\alpha 1$	$\alpha 2$	$\alpha 1$	$\alpha 2$
0.2	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.85	0.85
			0.80	0.80	0.81	0.81	0.81	0.81	0.86	0.86
0.4	0.60	0.60	0.60	0.60	0.63	0.62	0.62	0.62	0.72	0.71
			0.60	0.60	0.63	0.63	0.63	0.63	0.73	0.73
0.5	0.50	0.50	0.51	0.50	0.54	0.53	0.54	0.52	0.65	0.64
			0.51	0.50	0.54	0.54	0.54	0.54	0.66	0.66
0.6	0.50	0.40§							0.59	0.57
									0.60	0.60
0.8	0.50	0.20§							0.50	0.42
									0.50	0.46

per cent, usually slightly. For the four cases of Table 4, the frequency of females in dioecious populations is 0.506, 0.513, 0.531 and 0.567, respectively.

Conditions for trioecy

The values of $\alpha 1$ and $\alpha 2$ are not always the same, unlike the nuclear case. This means that for some values of the relative fertility of hermaphrodites compared with males ($\alpha 2 < \alpha \leq \alpha 1$), rare males increase in frequency but do not eliminate the hermaphrodites, i.e. trioecy is the reproductive system. These trioecious systems are stable: the sex ratio at equilibrium is the same whether the males or the hermaphrodites are the rare type at the beginning of the calculation. With the three-locus model of sex control, each sexual phenotype has the same frequency in *C1* and *C2*. For the two-locus model, as the introduction of the male restorer alleles of both cytotypes at the same time is quite unrealistic, we have verified that trioecy evolves when one of the male restorer alleles is introduced first. The conditions are exactly the same as those already found. When $\alpha > \alpha 1$, none of the male restorer alleles increases. When $\alpha \leq \alpha 2$, both male restorer alleles invade. When $\alpha 2 < \alpha \leq \alpha 1$, the first male restorer allele introduced invades the corresponding cytotype: the population is then composed of a dioecious cytotype and a gynodioecious cytotype; the second male restorer allele does not increase. The only exception is that when $\alpha < 0.5$ (e.g. in the last case of Table 4) the second male restorer allele increases but does not eliminate the corresponding hermaphrodite restorer allele: one cytotype is dioecious and the other one is trioecious or androdioecious. Thus, with the two-locus control of sex, trioecy can also be the reproductive system but not all sexual phenotypes are found in each cytotype.

Discussion

We show here that dioecy evolves in less restrictive conditions when the control of sex is nuclear–cytoplasmic compared with when the sex determination is nuclear. Another noticeable difference is that trioecious reproductive systems can be stable. The cytoplasmic polymorphism is maintained under these reproductive systems.

The results presented here were obtained by comparing, in each calculation, male individuals, female individuals and one kind of hermaphrodite individual (i.e. characterized by fixed relative female and male fertilities). The results thus give us the reproductive system obtained when hermaphrodites in the population have a fixed sexual allocation and are in competition with wholly male individuals. Selfing in hermaphrodites was not considered in those calculations because we wanted to focus on the consequences of a change in the genetic control of sex. Selfing changes the conditions for the evolution of dioecy under any genetic control of sex but there is no reason to believe that selfing would change the direction of the effects of the sex determination. Compared with the stable reproductive system obtained when the control of sex is nuclear, the nuclear–cytoplasmic models more often give gynodioecy, dioecy and trioecy at the expense of hermaphroditism or androdioecy. Reproductive systems with females are thus obtained in a wider range of conditions, as could have been predicted. The important result here is that the cytoplasmic genes cause an increase in female frequency compared with purely nuclear control of sex and that, despite the fact that cytoplasmic genes play a part in the sex determination, this can select for maleness. This is proven by the fact that reproductive systems with males, dioecy or trioecy sometimes replace other reproductive systems, including gynodioecy. Furthermore, not only can male-sterile cytoplasmic mutations result in selection for females and afterwards males but nuclear–cytoplasmic polymorphism can be maintained under these new reproductive systems (dioecy and trioecy). It is thus possible that nuclear–cytoplasmic determination acts not only as a transient stage as has been previously proposed (Delannay *et al.*, 1981; Gouyon & Couvet, 1985) but can also be maintained during and after the evolution of dioecious reproductive systems.

If the alleles restoring male fertility have only a slight negative pleiotropic effect on fertility, the range of values in which dioecy is the stable reproductive system under nuclear–cytoplasmic control of sex is only a little larger than when the control of sex is nuclear. This nevertheless proves that dioecy can evolve from nuclear–cytoplasmic gynodioecy, contrary to the common belief (Ross, 1978; but see Maurice *et al.*, 1993). Two points can be made. Firstly, the evolution of dioecy is not favoured when the cytotype(s) responsible for male sterility is (are) not restored, i.e. when the control of sex is strictly cytoplasmic (Charlesworth, 1989; Maurice *et al.*, 1993). We showed here that once the cytotypes are restored, the selection on males does not depend on the extent to which the cytotypes are restored (frequency of restorer alleles) but does depend on the increase in female frequency. There is thus a discontinuity between a purely cytoplasmic control of sex and a nuclear–cytoplasmic control. Secondly, if we compare populations with individuals having the same characteristics (relative fertilities), nuclear–cytoplasmic sex determination favours the evolution of dioecy compared with nuclear deter-

mination. But if we compare populations with the same frequency of females, nuclear determination is more favourable to the evolution of males than a nuclear–cytoplasmic model (Table 5); one must nevertheless remember that females need to have a higher relative fertility to reach a given frequency if the control of sex is nuclear.

Atlan *et al.* (1991) have found variability for sex allocation among hermaphrodites of the gynodioecious species *Thymus vulgaris*, which is known to have nuclear–cytoplasmic sex determination (Belhassen *et al.*, 1991). It is not known whether these differences result from different restorer alleles or from modifier genes acting on restored individuals. We thus considered both possibilities for the control of maleness: the two-locus model corresponds to the 'different restorers' hypothesis and the three-locus model corresponds to the 'modifiers' hypothesis. The results are very similar between the two models. Males are a little more easily selected in the three-locus model and trioecy more rarely obtained.

Few studies have been carried out on the evolution of dioecy under nuclear–cytoplasmic sex determination. Gregorius & Ross (1987) studied the evolution of dioecy in a model with two cytotypes and one nuclear locus with two alleles in which the nuclear allelic difference produces dioecy in one cytotype and hermaphrodism or gynodioecy in the other cytotype. They showed that dioecy can evolve by loss of the nondioecious cytotype. Their results cannot be compared with ours because in their model the reproductive system obtained is the result of the competition between the two cytotypes whereas in our model it is the result of the competition between hermaphrodite and male genotypes in each cytotype. Maurice *et al.* (1993) studied the evolution of dioecy in a two-cytotype–two-loci model based on the first model proposed for nuclear–cytoplasmic gynodioecy (Charlesworth, 1981; Delannay *et al.*, 1981), i.e. all individuals of one cytotype are male-fertile and individuals of the other cytotype are male-sterile (female) or male-fertile depending on the genotype at the nuclear restoration locus. In their model, as in the model presented here, both cytotypes can yield hermaphrodite and male individuals depending on the nuclear genotypes. They showed that male individuals, or a more male-biased sexual allocation in hermaphrodites, can evolve, but if selection towards maleness is too strong the cytoplasmic polymorphism is lost during the evolution of dioecy. The fact that nuclear–cytoplasmic gynodioecy can allow and even facilitate the evolution of dioecy thus seems to have some general value but whether that mode of sex control is retained in the new reproductive system depends on the particular sex determination system.

Table 5 Limit on the relative male fertility of hermaphrodites ($\alpha 2$) for the evolution of dioecy, for nuclear and nuclear–cytoplasmic sex determination models and for a given frequency of females in the gynodioecious population (F). For each model, the frequency of females in the population and the relative female fertility of hermaphrodites (β) are strictly inter-related. For the nuclear model, the relation is: $\beta = (1 - 2F)/2(1 - F)$ and the threshold value of α for the evolution of dioecy is given by the relation $\alpha + \beta = 1$ (Charnov *et al.*, 1976). For the nuclear–cytoplasmic model, the calculations are those for the three-locus model

	Control of sex			
	Nuclear		Nuclear–cytoplasmic $d_m = 0.20$ $d_f = 0$	
F	β	$\alpha 2$	β	$\alpha 2$
0.415	0.15	0.85	0.20	0.81
0.228	0.35	0.65	0.40	0.63
0.090	0.45	0.55	0.50	0.54
	Nuclear		Nuclear–cytoplasmic $d_m = d_f = 0.20$	
F	β	$\alpha 2$	β	$\alpha 2$
0.489	0.02	0.98	0.20	0.86
0.378	0.20	0.80	0.40	0.73
0.219	0.36	0.64	0.60	0.60

In this model we have considered only selection of males in gynodioecious populations with one kind of hermaphrodite. In reality, whether completely male individuals or hermaphrodites that are more male are selected depends on: (1) whether female-sterile mutations or mutations that only slightly modify the male/female allocation of hermaphrodites arise more easily, and (2) if slight modifications of the sexual allocation are the more common, the sexual allocation selected in hermaphrodites depends on the set of all relative fertilities possible for the hermaphrodites and on the characteristics of sex determination, including the characteristics of cytotypes and the deleterious effects of restorers.

Nuclear–cytoplasmic interactions allow females and sometimes males or more male-biased hermaphrodites to be maintained. This can allow selection to act on these new sexual phenotypes (Gouyon & Couvet, 1985) and/or change the outcrossing rate of the population. If this occurs, a new reproductive system could be stable even if the nuclear–cytoplasmic polymorphism is finally lost. The consequences of nuclear–cytoplasmic interactions on reproductive systems are thus probably even more important than is shown here.

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Appendix: evolution of genotype frequencies

Two-locus model for the control of sex

Notations are the same as in the text and in Table 1. Deleterious effects of restorer alleles have the same value for *R1* and *R2* ($d_m1 = d_m2 = d_m$, $d_f1 = d_f2 = d_f$) and for *R'1* and *R'2* ($d_m'1 = d_m'2 = d_m'$, $d_f'1 = d_f'2 = d_f'$).

The relative frequencies of genotypes in ovules at the next generation are:

for ovules *C1r1r2*: $[C1r1r1r2r2 + \frac{1}{2}(1-d_f)\ C1r1r1R2r2 + \frac{1}{2}(1-d_f')\ C1r1r1R'2r2] + \beta.\frac{1}{2}(1-d_f)[C1R1r1r2r2 + \frac{1}{2}(1-d_f)\ C1R1r1R2r2 + \frac{1}{2}(1-d_f')\ C1R1r1R'2r2]$;

for ovules *C1R1R'2*: $\beta(1-d_f)(1-d_f')[\frac{1}{4}.C1R1r1R'2r2 + \frac{1}{4}.C1R1r1R'2R2 + \frac{1}{2}.C1R1r1R'2R'2 + \frac{1}{2}.C1R1R1R'2r2 + \frac{1}{2}.C1R1R1R'2R2 + C1R1R1R'2R'2]$; etc.

Ovules *C1R'1.* and *C2R'2.* cannot exist because the individuals *C1R'1...* and *C2..R'2.* are male. That implies that genotypes *CiR'iR'i..* do not exist either.

The relative frequencies of genotypes in pollen at the next generation are:

for pollen *r1r2*: $\alpha.\frac{1}{2}(1-d_m)[CiRiririjrj + \frac{1}{2}(1-d_m)CiRiriRjrj + \frac{1}{2}(1-d_m')CiRiriR'jrj] + \frac{1}{2}(1-d_m')[CiR'iririjrj + \frac{1}{2}(1-d_m)CiR'iriRjrj + \frac{1}{2}(1-d_m')CiR'iriR'jrj]$, with $(i,j) = (1,2)$ and $(2,1)$;

for pollen *R1R'2*: $\alpha(1-d_m')(1-d_m)[\frac{1}{4}C1R1r1R'2r2 + \frac{1}{4}C1R1r1R'2R2 + \frac{1}{2}.C1R1r1R'2R'2 + \frac{1}{2}.C1R1R1R'2r2 + \frac{1}{2}.C1R1R1R'2R2 + C1R1R1R'2R'2] + \frac{1}{2}(1-d_m')^2[\frac{1}{2}.C1R'1R1R'2r2 + \frac{1}{2}.C1R'1R1R'2R2 + C1R'1R1R'2R'2] + (1-d_m)(1-d_m')[\frac{1}{4}.C2R1r1R'2r2 + \frac{1}{4}.C2R1r1R'2R2 + \frac{1}{2}.C2R1R1R'2r2 + \frac{1}{2}.C2R1R1R'2R2] + \frac{1}{2}(1-d_m')^2[\frac{1}{2}.C2R'1R1R'2r2 + \frac{1}{2}.C2R'1R1R'2R2]$; etc.

The frequencies of genotypes at the next generation are obtained by multiplying the frequencies of ovules and pollen and dividing them by the total ovule and pollen productions:

total ovules = $[Ciririrjrj + (1 - d_f)[CiririRjrj + CiririRjRj]$
$+ (1 - d_f')CiririR'j.] + \beta(1 - d_f)[CiRiririrjrj$
$+ CiRiRirjrj + (1 - d_f)[CiRiririRjrj + CiRiririRjRj$
$+ CiRiRiRjrj + CiRiRiRjRj] + (1 - d_f')[CiRiririR'j.$
$+ CiRiRiR'j.]]$;

with $(i,j) = (1,2)$ and $(2,1)$;

total pollen = $\alpha(1 - d_m)[CiRiririrjrj + CiRiRirjrj$
$+ (1 - d_m)[CiRiriRjrj + CiRiriRjRj + CiRiRiRjrj$
$+ CiRiRiRjRj] + (1 - d_m')[CiRiririR'j. + CiRiRiR'j.]]$
$+ (1 - d_m')[CiR'ririrjrj + CiR'iRirjrj$
$+ (1 - d_m)[CiR'iriRjrj + CiR'iriRjRj + CiR'iRiRjrj$
$+ CiR'iRiRjRj] + (1 - d_m')[CiR'iriR'j.$
$+ CiR'iRiR'j.]]$;

with $(i,j) = (1,2)$ and $(2,1)$.

Three-locus model for the control of sex

Notations are the same as in the text and in Table 2. The deleterious effects of restorer alleles have the same values for $R1$ and $R2$.

The relative frequencies of genotypes in ovules at the next generation are:

for ovules $C1r1r2m$: $[C1r1r1r2r2mm + \frac{1}{2}.C1r1r1r2r2Mm]$
$+ \frac{1}{2}(1 - d_f)[C1r1r1R2r2mm + \frac{1}{2}.C1r1r1R2r2Mm]$
$+ \beta.\frac{1}{2}(1 - d_f)[C1R1r1r2r2mm$
$+ \frac{1}{2}(1 - d_f)C1R1r1R2r2mm]$;

for ovules $C1r1R2M$: $(1 - d_f)[\frac{1}{4}.C1r1r1R2r2Mm$
$+ \frac{1}{2}.C1r1r1R2r2MM + \frac{1}{2}.C1r1r1R2R2Mm$
$+ C1r1r1R2R2MM]$; etc.

Ovules $C1R1.M$ and $C2.R2M$ cannot exist because the individuals $C1R1...M.$ and $C2..R2.M.$ are male. Genotypes $CiRiRi..MM$ do not exist either.

The relative frequencies of genotypes in pollen at the next generation are:

for pollen $r1r2m$: $\alpha.\frac{1}{2}(1 - d_m)[CiRiririrjrjmm$
$+ \frac{1}{2}(1 - d_m)CiRiriRjrjmm] + \frac{1}{2}(1 - d_m)$
$[CiRiririrjrjMm + \frac{1}{2}(1 - d_m)CiRiriRjrjMm]$,

with $(i,j) = (1,2)$ and $(2,1)$;

for pollen $R1r2M$: $(1 - d_m)[\frac{1}{4}.C1R1r1r2r2Mm$
$+ \frac{1}{2}.C1R1r1r2r2MM + \frac{1}{2}.C1R1R1r2r2Mm]$
$+ \frac{1}{2}(1 - d_m)^2[\frac{1}{4}.C1R1r1R2r2Mm + \frac{1}{2}.C1R1r1R2r2MM$
$+ \frac{1}{2}.C1R1R1R2r2Mm] + \frac{1}{2}(1 - d_m)^2[\frac{1}{4}.C2R1r1R2r2Mm$
$+ \frac{1}{2}.C2R1r1R2r2MM + \frac{1}{2}.C2R1R1R2r2Mm$
$+ C2R1R1R2r2MM]$; etc.

The frequencies of genotypes at the next generation are obtained as above.

total ovules = $Ciririrjrj.. + (1 - d_f)CiririRj...$
$+ \beta(1 - d_f)CiRi.rjrjmm + \beta(1 - d_f)^2 CiRi.Rj.mm$;
with $(i,j) = (1,2)$ and $(2,1)$;

total pollen = $\alpha(1 - d_m)CiRi.rjrjmm$
$+ \alpha(1 - d_m)^2 CiRi.Rj.mm + (1 - d_m)CiRi.rjrjM.$
$+ (1 - d_m)^2 CiRi.Rj.M.$; with $(i,j) = (1,2)$ and $(2,1)$.

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Sex inheritance in gynodioecious species: a polygenic view

Bodil K. Ehlers[1,*], Sandrine Maurice[2] and Thomas Bataillon[3,4]

[1]*Department of Ecology and Genetics, University of Aarhus, Ny Munkegade, Building 540, 8000 Aarhus C, Denmark*
[2]*ISEM, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier, France*
[3]*UMR 1097 "Diversité et Génomes des Plantes Cultivées", Domaine de Melgueil, 34130 Mauguio, France*
[4]*BiRC, University of Aarhus, Høegh-Guldbergs Gade, Building 090, 8000 Aarhus C, Denmark*

Gynodioecy is defined as the coexistence of two different sexual morphs in a population: females and hermaphrodites. This breeding system is found among many different families of angiosperms and is usually under nucleo-cytoplasmic inheritance, with maternally inherited genes causing male sterility and nuclear factors restoring male fertility. Numerous theoretical models have investigated the conditions for the stable coexistence of females and hermaphrodites. To date, all models rest on the assumption that restoration of a given male sterile genotype is controlled by a single Mendelian factor. Here, we review data bearing on the genetic determinism of sex inheritance in three gynodiecious plant species. We suggest that restoration of male fertility is probably best viewed as a quantitative trait controlled by many loci. We develop a threshold model that accommodates an underlying polygenic trait, which is resolved at the phenotypic level in discrete sexual morphs. We use this model to reanalyse data in *Thymus vulgaris*, *Silene vulgaris* and *Plantago coronopus*. A simple Mendelian inheritance of sex determinism is unlikely in all three species. We discuss how our model can shed additional light on the genetics of restoration and point towards future efforts in the modelling of gynodioecy.

Keywords: cytoplasmic male sterility; quantitative threshold model; sex determination

1. INTRODUCTION

Plants with gynodioecious breeding systems are composed of populations where hermaphrodites, bearing perfect flowers, coexist with females. Gynodioecy is a widespread condition found among many angiosperm families. The sex determination mechanism of females and hermaphrodites is crucial for understanding the evolution and maintenance of this sexual polymorphism. For most gynodioecious species, a combination of cytoplasmic genes causing male sterility (hereafter CMS) and nuclear restorer alleles determine the sex of an individual plant (reviewed in Charlesworth 1981). In short, female plants carry a CMS gene blocking the development of functional anthers, while hermaphrodites either do not have such a CMS gene or carry at least one nuclear restorer allele that restores male function. Different CMS genes can coexist within a species and even within a population (e.g. Van Damme & van Delden 1982; Koelewijn & van Damme 1995*a*; Manicacci *et al.* 1996), each with its own specific nuclear restorer allele(s). The molecular mechanism responsible for male sterility is still unknown, but CMS has been attributed to a (chimeric) open reading frame (ORF) in the mitochondrial genome (e.g. in *Zea mays*, *Brassica napus*, *Petunia* spp.). CMS associated genes are thought to be derived from portions of known genes fused with unknown sequences (Wise & Pring 2002). These new ORFs encode proteins that are believed to interfere with pollen production, causing male sterility. Nuclear restorers alter the expression of these CMS associated genes by modifying their transcripts and suppressing their deleterious effects on male function, thereby restoring male fertility (Schnable & Wise 1998; Bentolila *et al.* 2002; Wise & Pring 2002).

The important feature of such a sex determination system is that CMS genes are located in the mitochondria, and thus are usually maternally inherited (Reboud & Zeyl 1994). Therefore, a CMS gene can invade a population of hermaphrodites provided that females who carry it have even a slight female fitness advantage compared to the female fitness of hermaphrodites (Lewis 1941). Higher seed set in females than hermaphrodites is indeed often observed (e.g. review in Delph 1999). For the females to be maintained in the population, however, polymorphism at nuclear restorer loci is needed, as the fixation of a restorer allele would cause the female phenotype to disappear. Evidence of 'hidden' CMS genes, masked by the fixation of nuclear restorers is found in many crop species where CMS is recovered after crosses between different lines or species for example, maize, rice (*Oryza sativa*) and sunflower (*Heliantus annuus*) (Schnable & Wise 1998). Maintenance of a polymorphism for nuclear restorer alleles is, however, possible if one assumes a cost of carrying such an allele in an alien cytoplasm (e.g. Charlesworth 1981; Frank 1989; Gouyon *et al.* 1991). A nuclear restorer allele is then selected for when in combination with the cytoplasm it restores, and selected

* Author for correspondence (bodil.ehlers@biology.au.dk).

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against otherwise. This generates frequency-dependent selection on both the CMS and nuclear restorer alleles. Empirical evidence for costs of restoration is still sparse but the existence of costs have been demonstrated in *Plantago lanceolata* (de Haan *et al.* 1997) and *Lobelia siphilitica* (Bailey 2002).

Most theoretical studies on the maintenance of nucleo-cytoplasmic gynodioecy have made the simplifying assumption that, despite variation in CMS genes, each CMS only needs one restorer allele in order to have its male function restored, and in its simplest form the nuclear restorer allele is assumed to be dominant to the maintainer allele (Charlesworth 1981; Gouyon *et al.* 1991; see Bailey *et al.* 2003 for the study of other patterns of dominance and Saur Jacobs & Wade 2003 for a recent review of the theory). However, empirical studies show that some CMS genes may need a combination of two or more nuclear restorer alleles in order to have their male function restored. For example, in maize the T-cytoplasm causing male sterility is restored by the joint action of the nuclear restorer allele *Rf2* in combination with one to three additional restorers (Schnable & Wise 1998; Wise *et al.* 1999). Koelewijn & van Damme (1995*b*) suggested that at least five restorer loci were involved in restoring male sterile cytoplasms of *Plantago coronopus*, and Dudle *et al.* (2001) found that sex determination in *L. siphilitica* seemed to vary among cytoplasms with one CMS gene being restored by a single dominant allele, while restoration of other CMS only could be explained by the action of several nuclear loci and/or epistatic effects.

Here we propose that several loci with additive effects may be involved in restoring a single CMS. The idea emerged when reanalysing data of experimental crosses of the gynodioecious *Thymus vulgaris* from Assouad (1972). Sex determination in *T. vulgaris* is cyto-nuclear. In *T. vulgaris* females, flowers vary in their anther development. Three different flower types, so-called 'b-', 'c-' and 'd-'females, can be distinguished depending on the development of the sterile anthers (Thompson *et al.* 2002). A female plant carries only one type of flower. Such flower polymorphisms have also been reported from other gynodioecious species and have been proposed to be simply due to the action of different CMS genes blocking the development of anthers at different stages (e.g. Van Damme & van Delden 1982). Thus female offspring of a female plant should all have the same flower type as their maternal plant, as they all carry the same cytoplasm. However, analysis of female offspring from the three types of in *T. vulgaris* shows that although the maternal flower type is the commonest phenotype in the female offspring, a female can produce female offspring with all three flower types. Moreover, the sex ratio in the offspring also varies among females of different flower types (figure 1). When pollinated by a single pollen donor, b-females produce more hermaphrodites than c-females (Fischer's exact test, $p<0.0001$) which in turn produce more hermaphrodites than d-females (Fischer's exact test, $p<0.0001$; see figure 1). The same pattern is also found in open pollinated b-, c- and d-females (data not shown). These results show that (i) females of *T. vulgaris* vary in their restoration ability and (ii) that the female types which morphologically resemble hermaphrodites most (type b) also have a higher proportion of hermaphrodites in their offspring compared to females whose flowers have

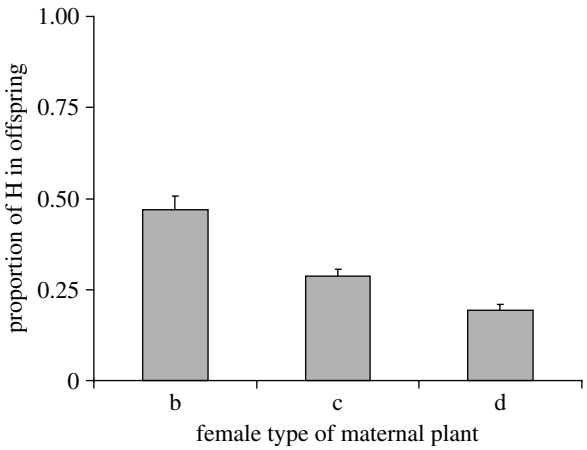

Figure 1. Mean proportion of hermaphrodites (±s.e.) in offspring of b-, c- and d-females (see text for further explanation) of *Thymus vulgaris*. Proportions are based on pooled offspring from 10 b-females ($n=344$ offspring), 17 c-females ($n=764$ offspring) and 18 d-females ($n=704$ offspring). Several pollen donors were used for these crosses, but each female was only crossed to a single pollen donor. Data extracted from Assouad (1972).

only little or no male organ vestiges. Gigord *et al.* (1999) also found that females with bigger corollas, which morphologically resemble hermaphrodites most, have a higher percentage of hermaphrodites in their progeny. The morphological gradation from d- (females with no male organs) to b-females (hermaphrodite-like) suggests that the restoration could be viewed as a quantitative trait controlled by several nuclear loci.

Below, we develop a threshold model for sex determination in gynodioecious species. We reanalyse data sets consisting of controlled crossing experiments from three different gynodioecious species (*P. coronopus*, *T. vulgaris* and *Silene vulgaris*). We show that our model can satisfactorily explain sex inheritance in such crosses. Finally, we discuss the value of our model and how it differs from Mendelian approaches adopted so far.

2. A THRESHOLD MODEL FOR SEX INHERITANCE

Threshold models have been widely used to model traits that are discrete at the phenotypic level but thought to be governed by polygenic inheritance. Traits whose inheritance have been successfully modelled through that framework include disease susceptibility, winglessness in insects (see Roff 1996 for a review) or more recently different sexual morphs (euphallic versus aphallic individuals) in snails (Ostrowski *et al.* 2000). Here, we assume that the discrete phenotypes (hermaphrodites and females) are controlled by an underlying trait, which we refer to as 'maleness'. Note that this trait is not actually observable. We assume that maleness is controlled by a large number of loci with additive gene action and is normally distributed in a population. The fraction of individuals that have a trait value higher than a given threshold, T, will have a hermaphrodite phenotype, whereas the remaining individuals will have a female phenotype (see figure 2*a*). Below, we outline our model for the case of a binary phenotype (female versus hermaphrodite), but the model can easily be extended to cases involving more than two phenotypes (see appendix).

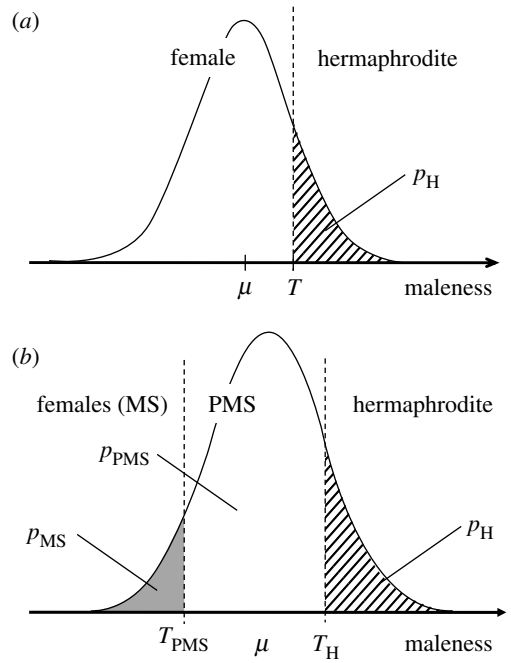

Figure 2. Graphical representation of the threshold model. (*a*) A two-morph threshold model with females and hermaphrodites. A single threshold T (dotted line) is used (*b*). A three-morph threshold model featuring females, partially male sterile (PMS) and hermaphrodites. Two thresholds, T_{PMS} and T_H, are used.

(a) *Predicted proportions of hermaphrodites in a cross*

We consider a cross between (i) a female (or a hermaphrodite maternal parent) carrying the CMS type i having genetic value M_F^i and (ii) a (hermaphrodite) male parent with genetic value M_H^i. These two parents are randomly sampled from a population where maleness is normally distributed. Given the assumptions above, an offspring from that cross will inherit a genetic value, G, which is normally distributed with mean $\mu = 1/2(M_H^i + M_F^i)$ and variance σ^2. The expected proportion of hermaphrodite phenotypes in such a cross is then

$$p_H = \int_T^{+\infty} \phi_G(x)\mathrm{d}x, \qquad (2.1)$$

where Φ_G is the probability density function of a Normal distribution with mean μ and variance σ^2.

(b) *Maximum likelihood estimation of the parameters of the threshold model*

A dataset (see below) will typically consists of a series of N crosses involving n male parents with genetic values $M_{H1}^i, M_{H2}^i, \ldots, M_{Hn}^i$ crossed to a series of m female parents with values $M_{F1}^i, M_{F2}^i, \ldots, M_{Fm}^i$. Let us 'label' the crosses $c_1, c_2, \ldots, c_N$. Within the jth cross we observe nf_j females and nh_j hermaphrodites. If we assume that the threshold model outlined above governs inheritance of sexual phenotypes in such a cross, we can write the likelihood of such data as

$$\ell(c_j) = \binom{nf_j + nh_j}{nh_j} p_H^{nh_j}(1 - p_H)^{nf_j}, \qquad (2.2)$$

where p_H is the probability defined by equation (2.1). The likelihood of the whole dataset is then (assuming N independent crosses)

$$\ell_D = \prod_{j=1}^{N} \ell(c_j).$$

Maximum likelihood estimates (hereafter MLE) of the parameters of the model can be obtained by maximizing ℓ_D over the space of parameters. The parameters are the genetic values of the different parents involved in the crosses, i.e. M_F's and M_H's values, the within-family variance σ^2 and the value of the threshold T. Our focus here is predicting the sex ratio of progeny using our threshold model and not to estimate components of variance or the heritability of the maleness trait. We will restrict our analysis to crosses involving unrelated individual resulting in progeny that are assumed to be outbred, and thus assume that within-family variances are identical for a given species dataset. Without loss of generality we can set the within-family variance to $\sigma^2 = 1$ and set the threshold to $T = 0$. Note that assuming a different value for T would only shift all genetic values accordingly but would not change the predicted sex ratios under the threshold model. If only a single cross was available as data, the genetic value of the mother, M_{F1} and the father M_{H1} of the cross would not be estimable, only their sum would (as it determines the mean of the distribution of within-family values for maleness). But as data from more independent crosses involving the same parents are available, all parental genetic values can be estimated (§3 for details on the type of crosses analysed for each species) provided that at least k crosses are available (if k parental genetic values are to be estimated). Note, however, that some maternal effects may be embedded into the maternal genetic value we estimate.

The likelihood of the data under the threshold model can be compared with a saturated model fitting the data perfectly. The saturated model is obtained by setting in equation (2.2) the ps to their observed value for each cross. Our threshold model is nested within this saturated model, and a likelihood ratio test, also known as a G-test, can therefore be used to assess the fit of our model relative to the saturated model. A large value of G indicates that the saturated model fits the data significantly better and that some of the variation in sex ratio is not accounted for by the threshold model. The G-statistic is distributed as a chi-square with d degrees of freedom, where d is the number of parameters fitted in the saturated model minus the number of parameters used by the threshold model. All calculations were carried out using MATHEMATICA 4.1 (Wolfram 1996) and likelihoods were maximized numerically using the function FindMinimum.

3. DATA ANALYSIS

(a) *Plantago coronopus*

The data of Koelewijn & van Damme (1995*b*) consist of controlled crosses made between plants sampled at two locations, 'KwadeHoek' (hereafter KH) and Oostvoornse meer (hereafter OM). Within each location, two ecologically different sites were surveyed: (i) KH, a dune a salt marsh and (ii) OM a dune and a meadow. At each site, three hermaphrodites and two females were sampled within an area of about 50 m^2. The following crosses were made (omitting data from selfing hermaphrodites that are not meeting the assumption of outbred progeny of our model).

Each female was crossed with every hermaphrodite used as a male parent resulting in 6 sets of offspring.
Hermaphrodites were crossed with each other (including reciprocal crosses) resulting in 6 sets of offspring.

In total, there were thus 12 sets of crosses per site involving five different parents. The data used here are extracted from the subtables 'first generation crosses' of tables 1–4 from Koelewijn & van Damme (1995*b*). The maternal plants have two possible cytoplasms, which are, respectively, denoted by cytoplasm 1 and 2. Within each set of offspring, three categories of individual were recorded: (i) male sterile (MS or females), (ii) partially male sterile (PMS) and (iii) hermaphrodites (H). The categories PMS and H were pooled in a single category (as done by the authors in the original paper).

The threshold model was fitted by estimating eight parameters (three genetic values of the hermaphrodites for each of the two cytoplasms and the two genetic values of the mothers). These genetic values were then used to predict sex ratios within each cross. Our threshold model predicts well the proportion of hermaphrodites for all but a few crosses from the last sampling site (OM meadow; table 1). Accordingly, the saturated model does not the fit the data better than our reduced model in two of the four sites (table 1).

(b) *Thymus vulgaris*

We reanalysed data from the study of Belhassen *et al.* (1991), also analysed by Charlesworth & Laporte (1998) using several Mendelian models. The data consisted of crosses of nine female plants all descended from the same maternal plant, i.e. all carrying the same cytoplasm, crossed to six hermaphrodites from the same population as the female plants. Not all possible crosses among the 15 plants (nine females, six hermaphrodites) were performed but, each hermaphrodite was crossed with four of the nine females (see table 5 in Belhassen *et al.* 1991) and the sex ratio of the offspring was recorded. Using available data from all 24 independent crosses, we fitted our model by estimating the genetic value of female and hermaphrodite plants (one for each parent so 15 parameters in total). This allowed us to obtain the sex ratios predicted under the threshold model for comparison with the observed ones. The model predicts the sex ratio very well, except for crosses with very few offspring (table 2; cross c10 and c17). Over all, due to those crosses where predictions from the threshold model are off by a few percent, the saturated model fits the data better than our reduced model.

(c) *Silene vulgaris*

Charlesworth & Laporte (1998) analysed data from several crossing experiments involving individuals originating from two different populations of *S. vulgaris*. The data we could use to apply to the threshold model from that study was, however, limited as individuals had to involved in several different crosses in order to obtain be able to estimate their parental genetic value. Given these requirements, five crosses (crosses 18–22 from table 6 in Charlesworth & Laporte 1998) were used. Our threshold model was fitted using four parameters (one for each parent involved in the crosses). The threshold model provides a good fit between observed and expected proportion of hermaphrodites in all five crosses, and the saturated model does not fit the data significantly better (table 3).

Table 1. Observed (obs. H) and expected (exp. H) values of proportion of hermaphrodites in offspring of *Plantago coronopus* under the threshold model.
(Data was obtained from tables 1–4 in Koelewijn & van Damme (1995*b*); n, number of offspring in each cross; cross no. refers to the numbering used in the original presentation of the data. $G_{\text{threshold}}$ is the statistic comparing the fit of the threshold model relative to a saturated model fitting the data perfectly. Within each site, $G_{\text{threshold}}$ is distributed as a chi-square with 4 degrees of freedom (12 crosses minus eight parameters fitted in the threshold model).)

cross	exp. H	obs. H	n	$G_{\text{threshold}}$
site: KH-dune				
c107	0.999	0.977	43	4.401
c109	1	1	75	0
c108	0.978	1	75	3.337
c111	1	1	62	0
c110	1	1	79	0
c112	0.932	0.932	59	0
c35	1	1	70	0
c36	0.96	0.986	70	1.589
c37	0.472	0.437	71	0.358
c38	1	1	72	0
c39	0.802	0.806	72	0.006
c40	0.809	0.809	68	0
total				9.710 ($p=0.046$)
site: KH-salt march				
c101	0.998	1	66	0.264
c103	1	1	55	0
c102	1	1	59	0
c105	0.887	0.9	10	0.0175
c104	1	1	61	0
c106	0.769	0.768	181	0.001
c29	0.567	0.565	62	0.002
c30	0.38	0.382	68	0.002
c31	0.781	0.781	73	0
c32	0.833	0.833	72	0
c33	1	1	72	0
c34	1	1	70	0
total				0.287 ($p>0.5$)
site: OM-dune				
c83	1	1	76	0
c85	0.897	0.865	133	1.385
c84	1	1	66	0
c87	1	1	78	0
c86	0.665	0.7335	75	1.634
c88	1	1	64	0
c23	0.908	0.971	68	4.237
c24	0.45	0.45	60	0
c25	0.688	0.612	67	1.737
c26	0.944	0.944	72	0.0003
c27	0.185	0.185	54	0
c28	0.531	0.531	64	0
total				8.99 ($p=0.06$)
site: OM-meadow				
c77	0.469	0.469	81	0
c79	0.976	1	69	3.352
c78	1	1	78	0
c81	0.967	1	62	4.161
c80	0.999	0.986	72	3.418
c82	0.999	0.987	76	3.317
c17	0.221	0.027	75	24.06
c18	0.973	1	71	3.89
c19	0.852	1	76	24.35
c20	0.682	0.826	69	7.367
c21	0.999	1	75	0.150
c22	0.989	0.901	71	18.83
total				92.9*** ($p<0.001$)

Table 2. Observed (obs.) and expected (exp.) values of proportion of hermaphrodites in offspring of *Thymus vulgaris* under the threshold model.
(Data was obtained from Belhassen *et al.* (1991). Cross no. refers to the number of family numbers used Charlesworth & Laporte (1998); *n*, number of offspring in each cross. $G_{\text{threshold}}$ is the statistic comparing the fit of the threshold model relative to a saturated model fitting the data perfectly. $G_{\text{threshold}}$ is distributed as a chi-square with 9 degrees of freedom (24 crosses minus 15 parameters fitted in the threshold model).)

cross	exp. *H*	obs. *H*	*n*	$G_{\text{threshold}}$
c1	0.0235	0	44	2.093
c2	0.029	0.091	22	1.935
c3	0.021	0	9	0.382
c4	0.0346	0.031	32	0.011
c5	0.269	0.379	29	1.671
c6	0.333	0.268	41	0.802
c7	0.461	0.379	29	0.789
c8	0.362	0.412	34	0.358
c9	0.148	0.143	21	0.004
c10	0.195	0.286	7	0.332
c11	0.299	0.292	24	0.006
c12	0.218	0.2	20	0.039
c13	0.174	0.222	9	0.136
c14	0.031	0	41	2.582
c15	0.161	0.182	11	0.034
c16	0.257	0.290	31	0.176
c17	0.077	0.5	2	2.516
c18	1	0.922	51	7.678
c19	0.0698	0.072	57	0.0001
c20	0.127	0.097	31	0.275
c21	0.232	0	13	6.863
c22	0.059	0.091	44	0.699
c23	0.414	0.468	47	0.561
c24	0.319	0.289	38	0.155
total				30.10 ($p<0.001$)

Table 3. Observed (obs.) and expected (exp.) values of proportion of hermaphrodites in offspring of *Silene vulgaris* under the threshold model.
(Data obtained from Charlesworth & Laporte (1998; table 6). Cross no. refers to the family number used in Charlesworth & Laporte (1998); *n*, sample size in number of offspring. $G_{\text{threshold}}$ is the statistic comparing the fit of our threshold model relative to a saturated model fitting the data perfectly. $G_{\text{threshold}}$ is distributed as a chi-square with 1 degree of freedom (five crosses minus four parameters fitted in the threshold model).)

cross	exp. *H*	obs. *H*	*n*	$G_{\text{threshold}}$
c18	0.08	0.08	25	0
c19	0.799	0.75	24	0.340
c20	0.67	0.72	25	0.291
c21	0.08	0.12	25	0.477
c22	0.036	0	25	1.818
total				2.93 ($p=0.09$)

4. DISCUSSION

(a) *Patterns of sex inheritance in* Plantago, Thymus *and* Silene

In all three species, the models suggested by the authors as best fitting the observed sex ratio, all depart from a single Mendelian factor. A model with two loci was used to fit the *T. vulgaris* data (Charlesworth & Laporte 1998), three loci in the case of *S. vulgaris* (Charlesworth & Laporte 1998) and up to five loci were needed in the case of *P. coronopus* (Koelewijn & van Damme 1995*b*). All authors presented goodness of fit test (*G*-tests) to assess how well their Mendelian model of sex inheritance fitted the actual data. Accordingly, we also assessed the fit of our threshold model relative to a saturated model.

However, there is no simple way of comparing the fit of the threshold model with the Mendelian models used previously, because the two classes of models are not nested. Akaike's information criteria (Burnham & Anderson 1998) could potentially be used to compare these. But, such comparisons do not make sense here as the previous analysis performed by the authors actually proceeded by sequentially rejecting a number of simple Mendelian models, adding loci and/or dominance and epistasis until a satisfactory fit was achieved. It is then very hard to decide whether the last model retained fits the data robustly or if the achieved fit was partly due to chance. The procedure is best described by Koelewijn & Van Damme (1995*b*, p. 1771): 'Because the minimum overall model gave a bad fit, we have to assume the involvement of further (population specific) genes. All possible crosses can then be explained, but for every deviant cross a new locus has to be assumed. The number of genes involved thus increases rapidly, approaching polygenic determination of sex'. Instead of comparing the relative fits of the two classes of models we concentrate on the 'absolute' fit achieved by both types of approach.

Overall, assuming that restoration of cytoplasmic male sterility is a quantitative trait, does at least as good a job in explaining the data, in terms of absolute goodness of fit, as assuming that restoration is a classical Mendelian trait (compare observed and predicted proportions of hermaphrodites under the threshold model and *G*-tests in tables 1–3). In the *Plantago* data, the threshold model predicts accurately the observed sex ratios in three out of the four sites. In the last site (OM-dune), sex ratios predicted by the threshold model are off by 15–20% compared to the observed sex ratios in three of the 12 crosses where the Mendelian fits the data much better. This may be due to the fact that highly skewed sex ratios, very close to either 0 or 1, are not adequately captured by the Gaussian assumption of the threshold model when assuming homogeneous within-family variance. In the *Thymus* data, ignoring crosses with very few offspring, observed sex ratios are accurately predicted. Finally, the threshold model fits the sex ratios in the *Silene* dataset well whereas Mendelian models performed rather poorly in two of the five crosses reanalysed here (see table 6 in Charlesworth & Laporte 1998).

(b) *Biological insights on the restoration of CMS*

The data from *P. coronopus* consists of offspring from crosses involving two different cytoplasms. This gives us the opportunity to examine whether the restoration of cytoplasms 1 and 2 can be viewed as two different traits. We built two nested models that make different assumptions regarding restoration of male fertility. Model 1 assumes that the genetic value of a father changes with the cytoplasm into which his pollen is applied—this may be a reasonable assumption if the nuclear genes have different restoration effects depending on the cytoplasm it is crossed

Table 4. Likelihood of *Plantago* datasets under two competing threshold models for sex inheritance.
(l_1, likelihood of data under threshold model 1 which was fitted using eight free parameters (two genetic values for each of the three hermaphrodite and two genetic values for the females). l_2, likelihood of data under threshold model 2 which was fitted with five free parameters (one genetic value for each parent). G: log likelihood ratio test for comparisons of model 1 and 2. G is asymptotically distributed as a chi-square with three degrees of freedom.)

dataset	l_2	l_1	G
KH-dune	1.974×10^{-8}	8.993×10^{-14}	24.6 ($p<0.0001$)
KH-meadow	3.810×10^{-6}	1.657×10^{-11}	24.7 ($p<0.0001$)
OM-dune	9.108×10^{-10}	1.839×10^{-11}	7.8 ($p=0.05$)
OM-meadow	1.19×10^{-28}	4.389×10^{-42}	61 ($p<0.0001$)

with. Model 2 is nested within model 1 and assumes that the genetic value of a father is the same no matter which cytoplasm the pollen is crossed with. Table 4 summarizes the likelihood of the *P. coronopus* data under the two competing models. The G-statistic reveals that in three sites out of four, the model 1 fits the data far better than the alternative. In the fourth sampling site, the reduced model that assumes a single genetic value for each father fits the data extremely well, leaving little room for improvement using model 1.

The estimated genetic values of hermaphrodites on cytoplasm 1 show no correlation with those of the same hermaphrodites on cytoplasm 2 ($r=0.041$, $p>0.1$). If a positive correlation was found, hermaphrodites good at restoring cytoplasm 1 should also be good at restoring cytoplasm 2 and thus the restoration of the two cytoplasms could be regarded as a single trait. The absence of a correlation between the genetic values, suggest that restoration of the two cytoplasms may be viewed as two independent traits. Note that sampling errors around the estimates of genetic values may reduce the power to detect correlations between the two traits. However, comparison of the relative fit of model 1 versus model 2 (table 4; LRT) indicates that the full model is often much better at explaining the data. This supports the idea the that ability to restore cytoplasm 1 is indeed independent of the ability to restore a different type of cytoplasm 2.

Our threshold model can also be used to estimate genetic values and thus characterize individuals with respect to their restoration ability. Such information is important in designing future crosses both in crop improvement where some breeding programmes routinely use cytoplasmic male sterilities and to generate testable predictions to investigate further patterns of sex inheritance in gynodioecious species.

(c) *Extending the threshold model to incorporate intermediate morphs*

In many gynodioecious species intermediate forms are reported. In *T. vulgaris* the b-type female could represent such an intermediate form. In *Beta vulgaris* two types of females coexist, the female type with more developed stamens has, like in *Thymus*, more hermaphrodites in its progeny (Boutin-Stadler *et al.* 1989). In *Plantago* species partial male steriles (PMS) are often reported in significant numbers (e.g. van Damme & van Delden 1982, Koelewijn & van Damme 1995*b*), and in *S. vulgaris* both PMS and female plants with different levels of anther development have been found (Charlesworth & Laporte 1998). The significance of these intermediate morphs for the evolution of cytoplasmic male sterility has not yet been investigated in detail, and they are usually just pooled with the female or the hermaphrodite morph in the analysis of crosses. Our threshold model provides a natural framework to take into account such morphs. The extension of the threshold model from two morphs to three morphs is straightforward (see appendix). Briefly, we still use a single underlying trait (termed maleness) but use two thresholds. For a maleness measure lower than the first threshold, the male sterile phenotype will be produced. For maleness between this and the next threshold, PMS will be observed and hermaphrodites correspond to individuals with maleness above the second threshold (see figure 2*b*). We used Koelewijn & van Damme's data on *P. coronopus* for the number on intermediates and analysed the data under the three morph threshold model. The model predicts well the proportion of females, hermaphrodites and intermediates (see appendix).

In conclusion, we have developed a threshold model to study sex inheritance in gynodioecious species. Use of that model to analyse patterns of inheritance in three different species show that viewing restoration of cytoplasmic male sterility as a quantitative trait is an alternative approach to understanding the often complicated restoration patterns of cytoplasmic male sterility. Moreover, the threshold model also allows us to deal with the PMS which are largely neglected when fitting Mendelian inheritance models.

Viewing restoration of male sterility as a polygenic trait calls for further research examining how different genetic determinism of sex inheritance shapes selection on CMS and restorer alleles. Models of the maintenance of gynodioecy have assumed so far that there are large fitness differences between sexual morphs and strong frequency dependent selection on nuclear restorer alleles. Alternative models assuming that restoration is a gradual process where fitness gains and costs are spread over a relatively large number of loci throughout the genome could potentially lead to different dynamics dominated by weak selection.

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APPENDIX. EXTENSION OF THE THRESHOLD MODEL TO THREE MORPHS

Three ordered categories of sexual phenotypes are assumed: male sterile, partially male sterile and hermaphrodites. These categories correspond to the data of Koelewijn & van Damme (1995*b*).

Let p_H, p_{PMS}, p_{MS} be the probability of producing, respectively, a hermaphrodite, a partially male sterile

phenotype and a male sterile phenotype. That probability is a function of the mean value of the family for maleness as well as two thresholds T_{PMS} and T_H. Assuming that maleness is normally distributed with mean μ and variance σ^2, the expected proportion of each morph are

$$\left.\begin{aligned} p_H &= \int_{T_H}^{+\infty} \phi_G(x)\mathrm{d}x, \\ p_{PMS} &= \int_{T_{PMS}}^{T_H} \phi_G(x)\mathrm{d}x, \\ p_{MS} &= \int_{-\infty}^{T_{PMS}} \phi_G(x)\mathrm{d}x. \end{aligned}\right\} \quad \text{(A 1)}$$

Here ϕ_G is the density of a normal distribution with mean μ and variance σ^2.

If within the jth cross n_1 male sterile, n_2 partially male sterile and n_3 hermaphrodites are observed, the likelihood associated with that dataset is

$$\ell(c_j) = \frac{(n_1+n_2+n_3)!}{n_1!n_2!n_3!} p_{MS}^{n_1} p_{PMS}^{n_2} p_H^{n_3}, \quad \text{(A 2)}$$

where the ps are the probabilities defined by equation (A 1). The likelihood of the whole dataset is then (assuming N independent crosses)

$$\ell_D = \prod_{j=1}^{N} \ell(c_j). \quad \text{(A 3)}$$

As for the two morphs model, MLE of the parameters of the model can be obtained by maximizing ℓ_D over the space of relevant parameters (the parental genetic values and the thresholds).

cross		MS	PMS	H	*n*
c107	E	0.002	0.03	0.97	43
	O	0.02	0.21	0.77	
c109	E	0.001	0.02	0.98	75
	O	0	0.08	0.92	
c108	E	0.04	0.17	0.79	75
	O	0	0.15	0.85	
c111	E	0.04	0.17	0.79	62
	O	0	0.08	0.92	
c110	E	0.04	0.16	0.80	79
	O	0.0	0.14	0.86	
c112	E	0.06	0.21	0.73	59
	O	0.07	0.24	0.69	
c35	E	0.05	0.19	0.76	70
	O	0	0.31	0.69	
c36	E	0.08	0.23	0.69	70
	O	0.01	0.09	0.90	
c37	E	0.36	0.36	0.28	71
	O	0.56	0.21	0.23	
c38	E	0.02	0.12	0.86	72
	O	0	0.07	0.93	
c39	E	0.12	0.29	0.59	72
	O	0.19	0.16	0.65	
c40	E	0.21	0.34	0.45	68
	O	0.19	0.50	0.31	

Observed (O) and expected (E) proportions of male sterile (MS), partially male sterile (PMS) and hermaphrodite (H) individuals in *P. coronopus* at the KH-dune site under a three morphs model.

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The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms

Sandrine Maurice and Theodore H. Fleming

Maurice, S. and Fleming, T. H. 1995. The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms. – Oikos 74: 55–60.

Insufficient pollination can affect the reproductive output and the rate of outcrossing of individual plants. We use a phenotypic model to explore the effect of pollen limitation on the evolution of plant reproductive systems. Compared to situations without pollen limitation, we show that conditions for the stability of different reproductive systems can change under pollen limitation: hermaphrodites are maintained under a larger set of conditions at the expense of unisexual types, especially males. We also show that trioecy, i.e., coexistence of hermaphrodites, males and females, can be evolutionarily stable, which is not the case in the absence of pollen limitation.

S. Maurice, IREMIA, Université de la Réunion, 97489 St Denis Cedex 09, France (present address: Institut des Sciences de l'Evolution, Université de Montpellier II, 34 095 Montpellier Cedex, France). – T. H. Fleming, Dept of Biology, Univ. of Miami, Coral Gables, FL 33124, USA.

In their theoretical studies of sex-ratio evolution in gynodioecious populations, Lewis (1941) and Lloyd (1974) proposed that seed production in females compared to hermaphrodites can be affected by pollen limitation. Pollen limitation can reduce the relative seed production in females compared to hermaphrodites if the hermaphrodites are self-compatible and if selfing requires the action of the pollination agent (wind or pollinator) to a lesser extent than outcrossing. In animal-pollinated species, a lack of pollinators could also alter the relative fertilities of females and hermaphrodites if hermaphrodites are more attractive to pollinators than females.

Differences in pollinator activity have also been invoked to explain changes in reproductive systems within and between species. A decrease in pollinator activity, causing an increase in the selfing rate of hermaphrodites, could favour the evolution of sexual dimorphism (gynodioecy or dioecy) to enforce outcrossing (Heine 1937 in Delph 1990). Delph (1990) found that sexual systems in species of *Hebe* tend to change from hermaphrodism to dioecy with increasing altitude and changes in the pollinator fauna. The reverse can also be argued: it could be better to be a self-compatible hermaphrodite if there is a real lack of pollinators, even if inbreeding depression is strong. *Parahebe* species from high altitudes have indeed evolved autogamy (Garnock-Jones 1976 in Delph 1990). *Armeria maritima* loses its heterostylic incompatibility system and regains self-compatibility at high latitudes (Baker 1966). Bierzychudek (1987), after studying apomictic and sexual forms of *Antennaria parvifolia*, proposed that apomixis could be an adaptation to the absence of pollinators in self-incompatible taxa.

In order to examine the effect of pollen limitation on the evolution of plant reproductive systems, we include this phenomenon in a phenotypic model of gender selection. The severity of pollen limitation can depend not only on the efficiency of the pollination agent but also on the frequency of pollen producers in a population. Lewis (1941) thus proposed that the probability that ovules of

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females will be fertilised decreases when the frequency of non-pollen producers (females) increases in a population. Lloyd (1974) used the following function for that probability: $p = 1\text{-}f^x$, where f is the frequency of females in the population and x is the average number of pollinator visits a flower receives. We hypothesise here that pollen limitation is likely to reduce the number of outcrossed ovules not only in females but also in hermaphrodites. We consider a general model where selfing may also require some pollinator visitation and where the probability that a non-selfed ovule will be fertilised can take various forms as a function of the frequency of females. The model below is simplified in that the "a priori" selfing rate is considered fixed, i.e., this selfing rate is determined by the morphology and phenology of the species. The realised selfing rate, i.e., the number of seeds produced by selfing versus the total number of seeds produced, does depend on pollination intensity.

Model and calculations

Fitnesses of the sexual phenotypes

We follow Charnov et al. (1976) and Charnov (1982) in writing the fitnesses of the different sexual phenotypes and use the following notations:

α: relative pollen production of hermaphrodites compared to males.
β: relative seed production of hermaphrodites compared to females (in the absence of pollen limitation and inbreeding depression).
s: "a priori" selfing rate of hermaphrodites, i.e., in the absence of pollen limitation (the realised selfing rate depends on the intensity of pollen limitation and is equal to: $sp_s / [sp_s + (1\text{-}s)p_c]$).
d: inbreeding depression (fitness of an offspring produced by selfing compared to an offspring produced by outcrossing = $1\text{-}d$).
p_s, p_c: probability that a flower will be visited enough to produce selfed (s) or outcrossed (c) seeds.
f, m, h: frequencies of females, males and hermaphrodites in the population.
f_G, f_D: frequencies of females in gynodioecious and dioecious populations at equilibrium.
W_f, W_m, W_h: relative fitnesses of females, males and hermaphrodites.

The fitnesses of the different sexual phenotypes are:

$$W_f = p_c,$$

$$W_h = \beta[1-s)p_c + 2s(1-d)p_s] + \frac{\alpha[fp_c + h\beta(1-s)p_c]}{m+\alpha h},$$

$$W_m = \frac{fp_c + h\beta(1-s)p_c}{m+\alpha h}$$

We can see that only the ratio of the probabilities of receiving enough pollinator visits to outcross or to self affects the relative fertilities of the different sexual phenotypes. This ratio will be denoted p_f ($= p_c/p_s$) in general and p_0 when the frequency of females is 0.

As the fitnesses are relative, we can divide their expressions by P_c to obtain:

$$W_f = 1,$$

$$W_h = \beta\left[1-s+2s\frac{1-d}{p_f}\right] + \frac{\alpha[f+h\beta(1-s)]}{m+\alpha h},$$

$$W_m = \frac{f+h\beta(1-s)}{m+\alpha h},$$

where the pollination factor clearly appears.

Conditions for the stability of the different reproductive systems

The three sexual phenotypes can be combined to give hermaphrodism, gynodioecy (coexistence of females and hermaphrodites), androdioecy (males and hermaphrodites), dioecy (females and males) and trioecy (females, males and hermaphrodites). We searched for conditions of stability for each of these reproductive systems by calculating the conditions under which a new sexual phenotype will increase in a population with these reproductive systems. The method is the following: 1) at equilibrium, the fitnesses of the sexual phenotypes present in the population are equal; 2) a new sexual type will increase if its fitness (when this new sexual type is rare) is greater than the fitness of the present types. The different situations are:

Population dioecious, condition for an hermaphrodite to increase

In a dioecious population at equilibrium, $W_f = W_m$, which gives $m = f = ½$ and $W_f = W_m = 1$. An hermaphrodite will thus increase if $W_h > 1$, which gives:

$$\beta\left[1-s+2s\frac{1-d}{p_{f_D}}\right] + \alpha > 1; \qquad \text{(I)}$$

i.e., dioecy is stable if $\beta\left[1-s+2s\frac{1-d}{p_{f_D}}\right] + \alpha < 1.$

Population hermaphroditic, condition for a female to increase

A female increases in a hermaphroditic population if $W_f > W_h$, which gives:

$$\beta\left[1-s+s\frac{1-d}{p_0}\right] < ½. \qquad \text{(II)}$$

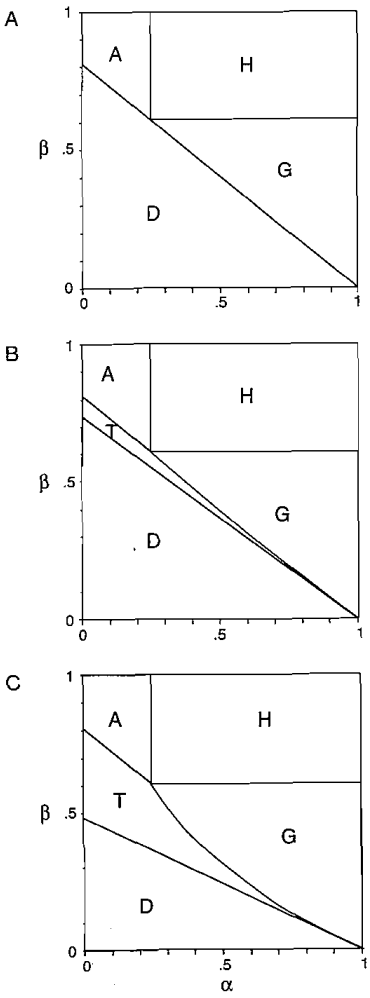

Fig. 1. Stable reproductive systems depending on the relative male (α) and female (β) fertilities of hermaphrodites, in the absence of pollen limitation (A), with a weak pollen limitation (B, $p_f = 1\text{-}f^3$) and with a strong pollen limitation (C, $p_f = 1\text{-}f$). The "a priori" selfing rate of hermaphrodites, s, is 0.6 and there is a weak inbreeding depression (d = 0.3). A: androdioecy, H: hermaphrodism, G: gynodioecy, T: trioecy, D: dioecy.

Population hermaphroditic, condition for a male to increase

$$W_m > W_h \text{ gives: } \alpha \frac{1-s+s\dfrac{1-d}{p_0}}{1-s} < \tfrac{1}{2}. \quad \text{(III)}$$

Population gynodioecious, condition for a male to increase

$W_f = W_h$ gives the frequency of females in a gynodioecious population as:

$$f_g = \frac{1-2\beta\left[1-s+s\dfrac{1-d}{p_{f_g}}\right]}{2\left[1-\beta\left[1-s+s\dfrac{1-d}{p_{f_g}}\right]\right]}.$$

$$W_m > W_h \text{ then gives: } \alpha+\beta\left[1-s+2s\frac{1-d}{p_{f_g}}\right] < 1. \quad \text{(IV)}$$

Population androdioecious, condition for a female to increase

$$W_h = W_m \text{ gives: } m = \frac{(1-s)-2\alpha\left[1-s+s\dfrac{1-d}{p_0}\right]}{2(1-\alpha)\left[1-s+s\dfrac{1-d}{p_0}\right]}.$$

$$W_f > W_m \text{ gives: } \alpha+\beta\left[1-s+2s\frac{1-d}{p_0}\right] < 1. \quad \text{(V)}$$

In order to have a stable trioecious reproductive system, each of the three sexual phenotypes must increase when rare. This is obtained when: a) hermaphrodites increase in a dioecious population, females increase in a hermaphroditic population and males increase in a gynodioecious population; i.e., when (I), (II) and (IV) are true, or b) hermaphrodites increase in a dioecious population, males increase in a hermaphroditic population and females increase in an androdioecious population, i.e., when (I), (III) and (V) are true.

In a trioecious reproductive system at equilibrium, $W_f = W_m = W_h$. Frequencies of the different sexual phenotypes are thus given by the following systems of equations:

$$\begin{cases} f+h\beta(1-s)=\alpha h+m \\ \beta\left[1-s+2s\dfrac{1-d}{p_f}\right]+\alpha=1 \\ f+h+m=1 \end{cases} \quad \text{or,}$$

$$\begin{cases} \beta[1-s+2s(1-d)/p_f]+\alpha=1 \\ 2f+h[\beta(1-s)+1-\alpha]=1 \\ f+h+m=1 \end{cases} \quad \text{(VI)}$$

In the absence of pollen limitation (i.e., p_f = 1), the system is:

$$\begin{cases} \beta[1+s(1-2d)]+\alpha=1 \\ f+h\beta(1-sd)=\tfrac{1}{2} \\ f+h+m=1 \end{cases} \quad \text{(VII)}$$

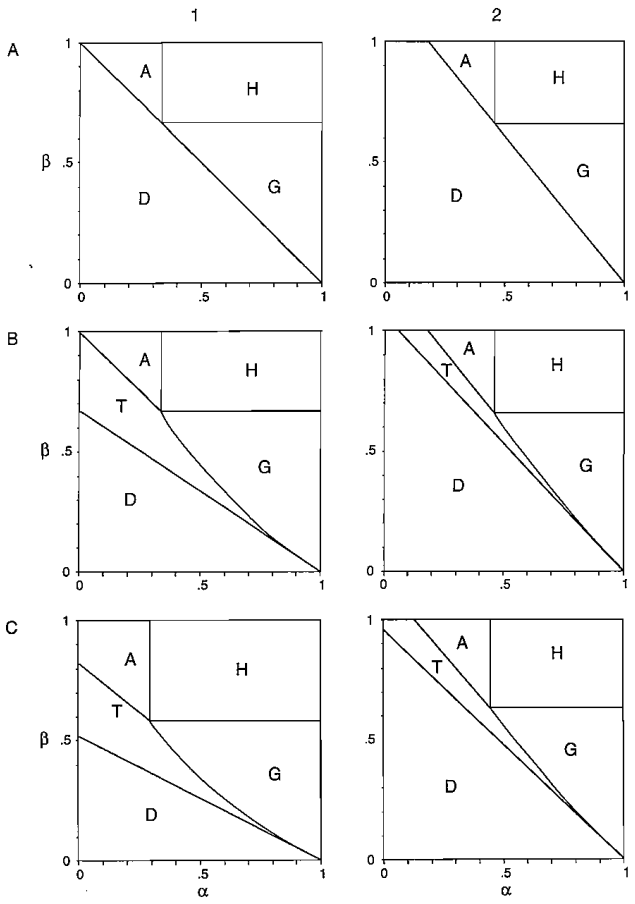

Fig. 2. Stable reproductive systems depending on the relative male (α) and female (β) fertilities of hermaphrodites, in the absence of pollen limitation (A), with pollen limitation in the presence of females only (B, $p_f = 1\text{-}f$), with pollen limitation even in the absence of females (C, $p_f = 0.7(1\text{-}f)$). In case 1, the "a priori" selfing rate of hermaphrodites, s, is 0.5 and the inbreeding depression, d, is 0.5. In case 2, $s = 0.3$ and $d = 0.8$. A: androdioecy, H: hermaphrodism, G: gynodioecy, T: trioecy, D: dioecy.

Examples

We have considered two kinds of functions to express pollen limitation:

1. The function proposed by Lloyd (1974), $p_f = 1\text{-}f^x$. In our model, this function implies that in the absence of females, selfing is as limited by pollinator activity as outcrossing. Examples of stable reproductive systems obtained under strong pollen limitation ($x = 1$), under weak pollen limitation ($x = 3$) and in absence of pollen limitation are illustrated on Fig. 1.

2. Because it is likely that outcrossing needs more pollinator visits than selfing, even in the absence of females, we also use the function $p_f = a\ (1\text{-}f^x)$, with $a < 1$ (Fig. 2).

Simplifications of the conditions above for these functions of pollen limitation are given in the Appendix. Examples of sex-ratios are given in Table 1.

Results and discussion

Changes in reproductive systems

Our calculations indicate that pollen limitation increases the range of conditions under which hermaphrodites are present in a population, even when inbreeding depression is strong (Fig. 1, 2, Table 1). This result will hold for any function of pollen limitation, as soon as outcrossing in more limited than selfing by the availability of pollen (i.e., $p_f < 1$, see the expressions for the relative fitnesses of the sexual types). The effect of pollen limitation increases when the "a priori" selfing rate of hermaphrodites increases and when the inbreeding depression decreases (see the expressions for the fitnesses and Fig. 2). The presence of hermaphrodites can lead to the coexistence of the three sexual phenotypes (see details below) or to the displacement of males (Fig. 1 and 2, panels B and C show an increase of the gynodioecy area at the expense of dioecy; Fig. 2, panels C show an increase of the hermaphrodism area at the expense of androdioecy and dioecy) and/or females (Fig. 2, panels C show an increase of the hermaphrodism area at the expense of gynodioecy and dioecy). When outcrossing and selfing are similarly pollen limited in the absence of females ($p_f = 1\text{-}f^x$, panels B and C of Fig. 1 and panel B of Fig. 2), conditions for the increase of a rare female strategy are obviously similar to those in the absence of pollen limitation. The areas of stability of hermaphrodism and androdioecy thus remain unchanged. When pollinators visitation is more limiting for outcrossing than for selfing even in the absence of females ($p_f = a\ (1\text{-}f^x)$, panel C of Fig. 2), then all areas of stability of the different reproductive systems are modified. In both cases, for a given set of parameters (a priori relative fertilities, a priori selfing rate, inbreeding depression), the stable reproductive system can change from dioecy to trioecy, and from trioecy to gynodioecy as pollination becomes more limiting (Fig. 1 and 2, Table 1).

The main effect of pollen limitation is to reduce the realised reproduction of unisexuals compared to hermaphrodites. The realised selfing rate of hermaphrodites is of course increased as some ovules fail to be outcrossed, but the difference between the realised selfing rate with (sa) and without (s) pollen limitation is usually not very large (Table 1). The selfing rate of hermaphrodites would be higher if ovules that failed to be outcrossed were then selfed. This hypothesis is not included in the calculations presented here. One can nevertheless affirm that, under this hypothesis, hermaphrodites would be even more favoured by pollen limitation. Results would thus not be qualitatively changed.

Maintenance of sexual polymorphism

Our calculations show that, for a subset of parameters, the three sexual phenotypes coexist in a population, i.e. trioecy is stable. In the absence of pollination limitation,

Table 1. Examples of stable reproductive systems and sex-ratios (frequencies of females (f), hermaphrodites (h) and males (m)) for different values of the selfing rate of hermaphrodites (s), the inbreeding depression (d), the function for pollen limitation and the relative male (α) and female (β) fertilities of hermaphrodites. The selfing rate achieved, $sa = s / (s + (1\text{-}s)p_f)$, has been calculated.

selfing rates		inbreeding depression	relative fertilities of hermaphrodites		pollen limitation	frequencies of sexual phenotypes			reproductive system
s	sa	d	α	β	p_f	f	h	m	
0.6	0.6	0.3	0.40	0.35	none	0.50	0	0.50	dioecy
	0.70				$1\text{-}f$	0.36	0.38	0.26	trioecy
	0.6		0.45	0.40	none	0.50	0	0.50	dioecy
	0.65				$1\text{-}f$	0.20	0.80	0	gynodioecy
	0.6		0.55	0.55	none	0.09	0.91	0	gynodioecy
	0.68				$0.7(1\text{-}f)$	0	1	0	hermaphrodism
0.5	0.5	0.5	0.50	0.40	none	0.50	0	0.50	dioecy
	0.60				$1\text{-}f$	0.33	0.48	0.19	trioecy
	0.64				$0.7(1\text{-}f)$	0.20	0.80	0	gynodioecy
0.3	0.3	0.8	0.50	0.55	none	0.50	0	0.50	dioecy
	0.43				$1\text{-}f$	0.42	0.17	0.41	trioecy

with or without selfing, the three sexual phenotypes will have equal fitnesses only if the relative fertilities of hermaphrodites compared to unisexuals are exactly on the line $\alpha+\beta(1\text{-}s\ (1\text{–}2d)) = 1$ (eq. VII), as already stated by Charnov et al. (1976) in the absence of selfing. For a given pair (α,β) on that line, the frequencies of the sexual phenotypes cannot be completely solved and a line of solution for the frequencies does exist (eq. VII). This explains why trioecy is not stable, even if the pair (α,β) meets the requirement, which is already improbable. Should the frequencies of sexual phenotypes be altered by drift or migration, selection will tend to bring them back to the line of equilibrium but not exactly at the same point. One sexual type (f, m or h) or the two unisexuals will eventually be lost. It can also be noted that a new sexual phenotype arriving in a population at equilibrium for the two other sexual phenotypes is at most (i.e., if the population is infinite) neutral and thus can increase only by chance.

In the case of pollen limitation and if the level of pollen limitation depends on the frequency of females, the three sexual phenotypes can have equal fitnesses for more than a line of (α,β) parameters. The conditions in (α,β) are linked to the frequencies of sexual phenotypes (eq. VI). For a given (α,β) in that set of solutions, trioecy is stable and the sexual frequencies are exactly determined.

Conclusions

Classical models of the evolution of reproductive systems show that, in the presence of inbreeding depression, an increase in the selfing rate of hermaphrodites favours the evolution of dioecy (Charlesworth and Charlesworth 1978). We show here that although a decrease in the abundance or efficiency of pollination agent increases the relative selfing rate of hermaphrodites, it does not favour the evolution of unisexuality. This is because reduced pollination affects the fitness of unisexual individuals even more strongly than it affects hermaphrodites. This situation supports Darwin (1877), who wrote: "As we must assume that cross-fertilisation was assured before an hermaphrodite could be changed into a dioecious plant, we may conclude that the conversion has not been effected for the sake of gaining the great benefits which follow from cross-fertilisation.". Rather than favouring the evolution of dioecy from hermaphrodism, reduced pollination could be responsible for evolution in the reverse direction: from dioecy to gynodioecy, androdioecy or hermaphrodism if the bisexual types reappear by mutation or migration. This has been proposed as an explanation for the androdioecious system of *Datisca glomerata* (Liston et al. 1990).

Dioecy has been said to occur more frequently in species pollinated by small, unspecialised insects (Bawa 1980, Givnish 1982). The evolutionary factor proposed is that these pollinators stay a long time on the same individual, causing high rates of selfing in hermaphrodites. We would like to emphasise that this argument can only be valid if the behaviour of pollinators is such that it reduces the outcrossing rate of hermaphrodites more than it reduces the reproduction of unisexuals. One can even say "much more" because in this "reverse hypothesis", ovules of hermaphrodites are selfed if they are not outcrossed whereas in our model, ovules of hermaphrodites are lost if they are not outcrossed.

Our model indicates that pollen limitation can be responsible for the coexistence of more than two sexual phenotypes in a population, either as an intermediate stage or as an equilibrium if the lack of pollen increases with the frequency of females in the population. Few cases of true trioecy, with separate female, male and hermaphrodite individuals, have been described. One example is the bat-pollinated columnar cactus *Pachycereus pringlei* which possesses gynodioecious and trioecious populations in the Sonoran Desert of Mexico. In this species, pollination has been shown to limit seed set in females but not in hermaphrodites and the presence of

males in populations is correlated with proximity to major bat roosts (Fleming *et al.* 1994). Pollen limitation thus appears to be a major factor in the evolution of the geographically variable breeding system of this species.

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Appendix

Conditions for the stability of the different reproductive systems, for two kinds of pollen limitation, p_f.

1. $p_f = 1\text{-}f^x$

Pollen limitation does not change the frequency of females ($^1/_2$) in dioecious populations.
Conditions become:

(I) $\beta\left[1-s+2s\dfrac{1-d}{1-½^x}\right]+\alpha>1$

(II) $\beta[1-sd]<½$

(III) $\alpha\dfrac{1-sd}{1-s}<½$

(IV) $\alpha+\beta\left[1-s+2s\dfrac{1-d}{1-f_G^x}\right]<1$

(V) $\alpha+\beta[1-sd]<1$

The frequency of females in a gynodioecious population is given by:

$f_G^{x+1}[-2(1-\beta(1-s))]+f_G^x[1-2(1-s)]+f_G[2(1-\beta(1-sd))]+2\beta(1-sd)-1=0.$

If $x = 1$, then $f_G = \dfrac{1-2\beta(1-sd)}{2[1-\beta(1-s)]}$.

For $x = 3$, calculations of f_G and condition (IV) have been made using Mathematica (Wolfram Co).

2. $p_f = a\ (1\text{-}f^x)$, with $a < 1$

We have made calculations only for $x = 1$.
The frequency of females in gynodioecious populations is:

$$f_G = \frac{1-2\beta\left[1-s+s\dfrac{1-d}{\alpha}\right]}{2[1-\beta(1-s)]}$$

and $p_0 = a$, $p_{f_0} = ½\ a$, $p_{f_G} = a(1-f_G)$.

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Geographic variation in the breeding system and the evolutionary stability of trioecy in *Pachycereus pringlei* (Cactaceae)

THEODORE H. FLEMING,[1]* SANDRINE MAURICE[2] and J.L. HAMRICK[3]

[1]*Department of Biology, University of Miami, Coral Gables, FL 33124 USA,* [2]*Laboratoire Genetique et Environnement, Institut des Sciences de l'Evolution, Université Montpellier II, F-34095 Montpellier Cedex 05, France and* [3]*Departments of Botany and Genetics, University of Georgia, Athens, GA 30602, USA*

Summary

The Sonoran Desert columnar cactus *Pachycereus pringlei* has a geographically variable, non-hermaphroditic breeding system. It is trioecious (separate males, females and hermaphrodites) in the northern two-thirds of its range in Sonora, Mexico, and in the southern three-quarters of its range in Baja California, Mexico, and is gynodioecious (separate females and hermaphrodites) elsewhere. Trioecy occurs near known maternity roosts of its major pollinator, the nectar-feeding bat *Leptonycteris curasoae*; gynodioecy occurs > 50 km from known bat roosts. The observed geographic patterns cannot be explained by limited gene flow or by the geographic distributions of diurnal avian pollinators. Our field observations plus a theoretical analysis suggest that the abundance of chiropteran pollinators plays an important role in the maintenance of trioecy in this plant. Under pollinator limitation, trioecy can be a stable breeding system in this species.

Keywords: bats; breeding systems; Cactaceae; gene flow; genetic diversity; gynodioecy; pollinator abundance; trioecy

Introduction

Non-hermaphroditic breeding systems are relatively uncommon in plants. Lloyd and Bawa (1984) estimated that only about 10% of all seed plants have breeding systems involving dioecism, gynodioecism or subdioecism. Geographic variation in sex ratios and in the occurrence of monomorphic versus dimorphic breeding systems has been noted in a number of dimorphic species. A particularly well-studied species is *Wurmbea dioica* (Liliaceae) in which populations in South Australia, Victoria and the Australian Capital Territory are dimorphic, while populations in Western Australia are either monomorphic or dimorphic (Barrett, 1992). Other examples of geographic variation in the breeding system of dimorphic plants include various species of apioid umbellifers in New Zealand (Webb, 1979), *Hebe strictissima* (Scrophulariaceae) in New Zealand (Delph, 1990), *Phacelia linearis* (Hydrophyllaceae) in Utah, USA (Eckhart, 1992) and *Echinocereus coccineus* (Cactaceae) in the Southwestern USA (Hoffman, 1992).

Factors associated with geographic variation in the breeding systems of non-hermaphroditic plants include variation in physical environmental conditions as well as pollinator availability. In Western Australia, for example, monomorphic populations of *W. dioica* occur at moist sites on rich soils; dimorphic populations occur at arid sites on shallow soils (Barrett, 1992). The frequency of females varies along environmental gradients in a number of gynodioecious species (e.g. Kessel and Jain, 1984; Van Damme and Van Delden, 1984; Delph, 1990). Within certain New Zealand

*Author to whom all correspondence should be addressed.

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umbellifers, females are less common and hermaphrodites set more fruit at higher altitudes and latitudes, apparently in response to reduced pollinator abundance and/or shorter flowering seasons (Webb, 1979). In a population where its normal pollinators (i.e. oligolectic pollen-collecting bees) are absent, *Nemophila menziesii* (Hydrophyllaceae) is gynodioecious, whereas other populations are hermaphroditic (Ganders, 1978).

Trioecy – the co-occurrence of males, females and hermaphrodites at relatively high frequencies in populations – is even less common than dimorphism in flowering plants. The giant Mexican columnar cactus, *Pachycereus pringlei* (cardon), is one of two cactus species with a trioecious breeding system (Fleming *et al*., 1994). Trioecy in the other species, the bee-pollinated *Opuntia robusta*, is thought to be a transitional stage in the evolution of dioecy from hermaphroditism (del Castillo, 1986). In this paper, we present observations on (1) geographic variation in the breeding system of *P. pringlei* throughout its range in Sonora and Baja California, Mexico, (2) the distribution of its major pollinator, the nectar-feeding bat, *Leptonycteris curasoae* (Chiroptera: Phyllostomidae), and (3) the results of a theoretical model which suggest that, under conditions of pollinator limitation, trioecy can be a stable reproductive system in this species.

To explain observed geographic variation in the breeding system of this cactus we examine two hypotheses. The 'limited gene flow' hypothesis states that the absence of female sterility allele(s) in some populations results from limited gene flow among populations. If this hypothesis is correct, substantial genetic subdivision among populations should occur at gene loci that are not associated with sex determination. The 'nocturnal pollinator abundance' hypothesis states that, since the fitnesses of males and females of *P. pringlei* are much more pollinator-dependent than that of hermaphrodites, males and females should be uncommon in populations experiencing low flower visitation rates by *Leptonycteris* bats. Geographic variation in the form of *P. pringlei*'s breeding system should therefore be associated with geographic variation in the abundance of these bats.

The study organism

Pachycereus pringlei is a night-blooming, autotetraploid cactus distributed in lowland deserts in coastal Sonora and in desert and thorn scrub habitats throughout much of Baja California (Shreve and Wiggins, 1964; Murawski *et al*., 1994) (Fig. 1). Its large white flowers are open for less than 18 h and produce copious amounts of nectar and pollen. They are visited primarily by *Leptonycteris* bats at night and by native and exotic bees and birds in the morning before closing. Pollinator exclusion and hand-pollination experiments at Bahia Kino, Sonora (site 4 in Fig. 1) indicate that bats account for about 89% of the fruit set in females and hermaphrodites and that fruit set in females, but not in hermaphrodites, is pollen-limited; the missing pollinators are likely to be bats (Fleming *et al*., 1996). The proportions of males, females and hermaphrodites in this population are 0.29, 0.43 and 0.25, respectively (Table 1); a few individuals (proportion = 0.03) are double-steriles. Although we do not yet know the genetic basis for sex determination in *P. pringlei*, the existence of double-steriles suggests that it probably involves at least two unlinked nuclear loci.

Detailed analysis of reproduction in *P. pringlei* indicates that: (1) the anthers of female flowers lack pollen; (2) the large ovary of male flowers lacks ovules; (3) hermaphrodites are self-compatible, the outcrossing rate as determined by multilocus estimates based on several allozyme loci of seedlings is 0.34, and fruit set is about 8% in the absence of pollinator visits; (4) inbreeding depression is very low in the progeny of hermaphrodites; and (5) males produce more flowers and pollen per night and per season, and females produce more fruits and seeds per season, than hermaphrodites (Fleming *et al*., 1994; Murawski *et al*., 1994). Based on annual pollen and seed production, the fertilities of males and females relative to that of hermaphrodites are 1.52 and 1.62, respectively. In the absence of pollen limitation, females produce about 3.1 times more seeds

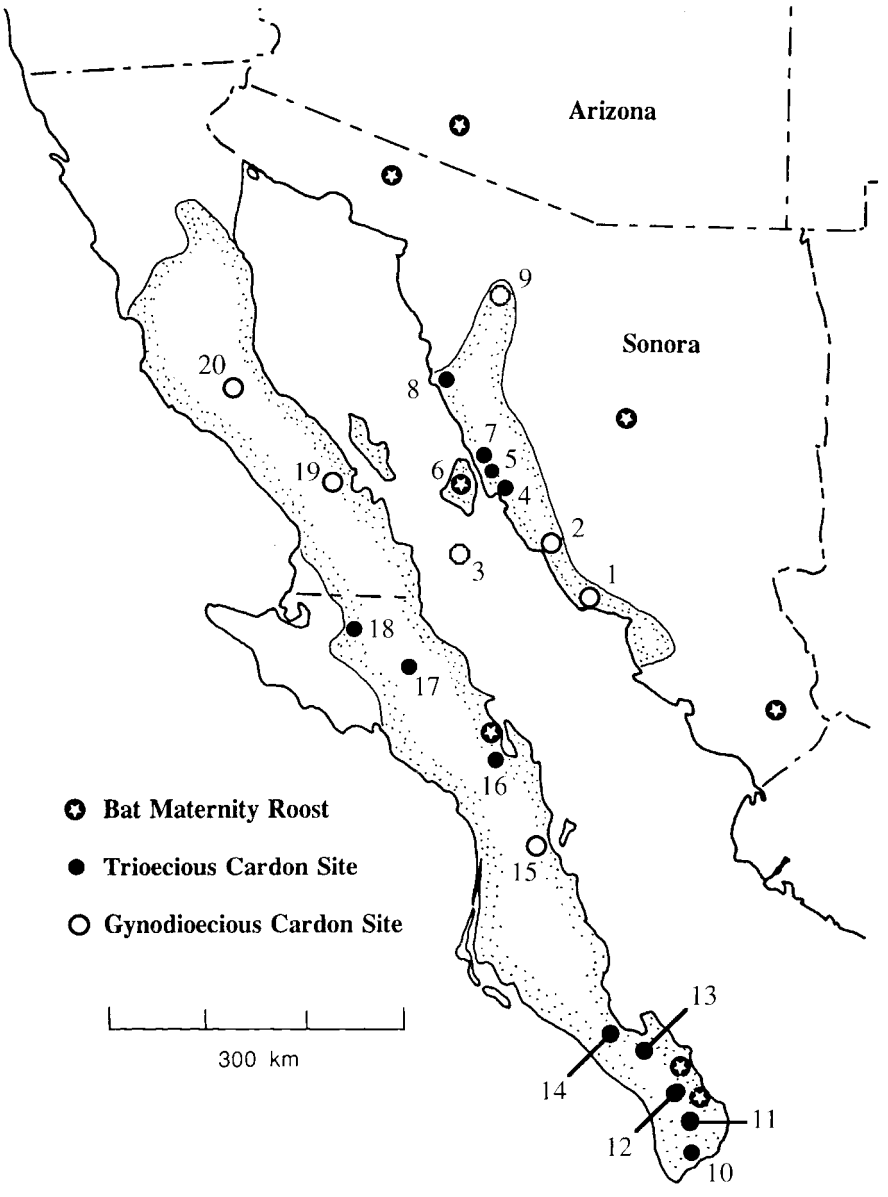

Figure 1. Map showing the location of *Pachycereus pringlei* study sites and *Leptonycteris curasoae* maternity roosts. Frequencies of different sex classes at each site can be found in Table 1.

annually than hermaphrodites. In this population, all flowers on all plants that we have examined are of one sexual type; examination of many individuals over a 6 year period revealed no changes in sexual type between years; and sexual type is independent of size (Fleming *et al.*, 1994 and unpublished).

A plot of functional femaleness in this population, calculated using equation (2) in Ross (1990), reveals a trimodal distribution with mean functional femaleness in hermaphrodites equalling 0.42 (S.E = 0.044, $n = 12$) (Fig. 2). We have no evidence of gender 'inconstancy' in males; no males at

Table 1. Frequencies of four sex classes in populations of *Pachycereus pringlei* in Sonora and Baja California, Mexico.

Site[a]	*n*	%Males	%Females	% Hermaphrodites	% Neuters
1. San Carlos, Son.	43	0	30.2	69.8	0
2. Tastiota Junction, Son.	48	0	33.3	66.7	0
3. Isla San Pedro Martir, Son.	93	0	46.2	53.8	0
4. Bahia Kino, Son.	211	28.4	41.7	28.0	1.9
5. Madrugada, Son.	47	42.6	38.3	19.1	0
6. Isla Tiburon, Son.	5	40.0	20.0	40.0	0
7. Punta Chueca, Son.	71	22.5	31.0	46.5	0
8. Puerto Libertad, Son.	59	35.6	39.0	25.4	0
9. Caborca, Son.	29	3.4	40.7	55.6	0
10. Cabo San Lucas, BCS	44	53.7	36.6	9.8	0
11. Santiago, BCS	16	68.7	31.3	0	0
12. Buena Vista, BCS	43	53.5	27.9	16.3	2.3
13. S of La Paz, BCS	20	75.0	20.0	5.0	0
14. W of La Paz, BCS	32	50.0	46.8	3.1	0
15. Loreto, BCS	37	0	29.7	70.2	0
16. Playa Cocos, BCS	33	30.3	36.4	33.3	0
17. W of Santa Rosalia, BCS	35	31.4	42.9	20.0	5.7
18. SE of Guerrero Negro, BCS	25	52.0	24.0	16.0	8.0
19. W of Bahia de Los Angeles, BCN	42	0	16.7	83.3	0
20. Catavina, BCN	34	0	44.1	55.9	0

[a] Numbers refer to the sites in Fig. 1. Except at Bahia Kino (site 4), data represent the frequencies observed on one day and may not represent true frequencies because hermaphrodites begin to flower somewhat later in the blooming season (by 1–2 weeks) than males and females (Fleming *et al*., 1994). Populations in Sonora were surveyed in mid-flowering season. Those in Baja California were surveyed early in the flowering season when the frequency of hermaphrodites may have been underestimated. Son. = Sonora, BCS = Baja California Sur, BCN = Baja California Norte.

Bahia Kino produce viable seeds. Hence, this species cannot be considered subdioecious, a more common breeding system than trioecy.

The geographic survey

To determine whether the breeding system of cardon varies geographically, we estimated sexual class frequencies in nine populations in Sonora and 11 populations in Baja California in April and May 1992 and in April 1993, respectively (Fig. 1). In each population, we examined one or more flowers per individual to determine its sexual class. In most populations, we examined flowers in the evening, just as they opened, when sexual class determination is unambiguous (Fleming *et al*., 1994).

The results of our survey revealed that in Sonora, populations south of Bahia Kino, including Isla San Pedro Martir, the most isolated island in the Gulf of California, lacked males and were gynodioecious (i.e. they contained only females and hermaphrodites) with an average hermaphrodite:female ratio of 1.7:1 (Table 1). Trioecious populations containing an average of 25.9% males occurred in the northern two-thirds of the range of *P. pringlei* in Sonora. Males occurred at low frequency at the far northern edge of its range.

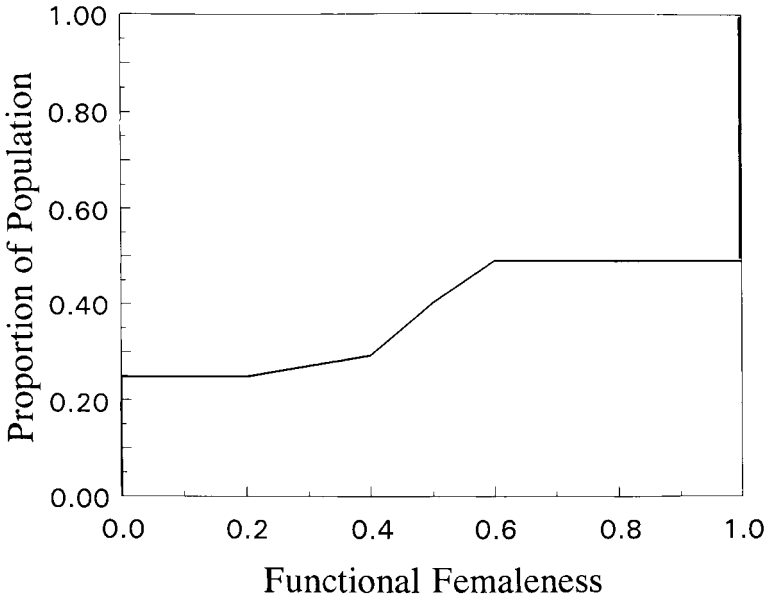

Figure 2. Variation in functional femaleness in a sample of 86 adults of *Pachycereus pringlei* examined in 1990 at site 4 in Fig. 1. Individuals with values of 0 and 1.0 are males and females, respectively. Functional femaleness of hermaphrodites was calculated using equation (2) in Ross (1990).

The geographic pattern in Baja California was the opposite of that in Sonora. With one exception (site 15), populations were trioecious in the southern three-quarters of the range of *P. pringlei* and contained up to 54% males in samples of more than 25 individuals (Table 1). North of 28°N, populations were gynodioecious and contained up to 83% hermaphrodites. In the entire dataset, male frequency was negatively correlated with frequency of hermaphrodites (arcsine-transformed data, $r^2 = 0.84$, $P < 0.001$). Female frequency was also negatively correlated with hermaphrodite frequency, but not significantly so (arcsine-transformed data, $r^2 = 0.038$, $P = 0.41$).

The 'limited gene flow' hypothesis

To assess whether geographic variation in the breeding system of *P. pachycereus* was associated with patterns of genetic variation among populations, we determined allele frequencies at 24 electrophoretically detectable allozyme loci, using one seedling from the fruit of 15–48 individuals in nine populations as a source of tissue. Seeds came from sites 2, 4 and 8 in Sonora and sites 10, 14, 15, 16, 19 and 20 in Baja California (Fig. 1).

The seeds were germinated in Petri dishes on moist filter paper, and seedlings were transplanted into normal greenhouse potting soil and placed in a greenhouse. When the seedlings were approximately 1 cm in length (i.e. 1 month old), each seedling was sliced into several pieces and, with the aid of liquid nitrogen and sand, was crushed to a fine powder with a mortar and pestle. An extraction buffer (Mitton *et al.*, 1979) was added to solubilize and stabilize the enzymes. Plant extracts were soaked onto chromatography paper wicks and stored at −70°C until analysis by starch gel electrophoresis.

Four buffer systems were used to resolve 11 enzyme systems on 10% starch gels. Buffer numbers refer to Table 1 in Soltis *et al.* (1983). System 6 was used for diaphorase (DIA), fluorescent esterase

(FE), alcohol dehydrogenase (ADH), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM). System 4 was used for 6-phosphogluconate dehydrogenase (6-PGDH), isocitrate dehydrogenase (IDH) and shikimate dehydrogenase (SKDH). System 7 was used for amino acid transferase (AAT) and triose phosphate isomerase (TPI). System 11 was used for malate dehydrogenase (MDH). Stain recipes are given in Soltis *et al.* (1983) except for AAT and DIA, which are given in Cheliak and Pitel (1984).

Since *P. pringlei* is an autotetraploid, each allozyme locus is duplicated. Due to the clarity of the allozyme banding patterns, we could distinguish heterozygous individuals with one, two or three copies of each allele. For our data analyses, we treated each pair of duplicated loci as a single locus. Our rationale for this was that the duplicated loci segregate tetrasomically (Murawski *et al.*, 1994) and thus function genetically as a single locus. Based on this decision, the 11 enzyme systems produced 24 loci. Genetic diversity parameters were calculated following Hedrick (1985) and Hamrick and Godt (1989). These measures were calculated for the species as a whole (subscript s), for each of the two regions (subscript r) and for each population individually (subscript p). The genetic parameters calculated include the proportion of polymorphic loci (P), the number of alleles per polymorphic locus (AP) and genetic diversity (H_e; where $H_e = 1 - \Sigma p_i^2$ and p_i is the frequency of the ith allele at a locus). We also partitioned the overall genetic diversity at each polymorphic locus (H_T) into components due to allele frequency differences between regions (D_r, Baja *vs* Sonora), among populations within regions (D_p) and within individual populations (H_S) using Nei's (1973) genetic diversity statistics. Nei's G_{ST}, the proportion of H_T due to differences among populations (or regions), was then calculated. This measure is the multiallelic equivalent of Wright's (1951) F_{ST} statistic. Separate analyses of the Baja and Sonoran populations allowed us to determine if the two regions differed in how they partitioned genetic diversity among populations. Indirect estimates of gene flow were obtained by $Nm = [(1 - G_{ST})/4G_{ST}]\alpha$ (Wright, 1931; Crow and Aoki, 1984), where Nm is the number of migrants per generation and α equals $[(n-1)/n]^2$ and is an adjustment for small population numbers (Crow and Aoki, 1984).

In the species as a whole, 22 of the 24 loci (92%) were polymorphic. Each polymorphic locus averaged 3.00 alleles and the overall genetic diversity (H_{es}) was 0.215 (Table 2). These genetic parameters generally varied little among regions or populations within regions. Across the six Baja populations, 83% of the loci were polymorphic, there were 2.85 alleles per polymorphic locus, and genetic diversity equalled 0.217. The pooled values for Sonora were somewhat lower, with $P_r = 0.75$, $AP_r = 2.50$ and $H_{er} = 0.199$. Seventeen alleles were unique to the Baja populations and seven alleles were unique to Sonora. The mean within-population genetic diversity parameters were $P_p = 0.67$, $AP_p = 2.44$ and $H_{ep} = 0.194$ for Sonora and $P_p = 0.65$, $AP_p = 2.52$ and $H_{ep} = 0.207$ for Baja. Thus, it appears that *P. pringlei* from Baja maintains marginally more genetic diversity than is found in Sonora. Baja populations of *P. pringlei* also have somewhat more among-population genetic diversity (4.0%) than do the Sonoran populations (2.4%). Overall, 5% of the total genetic diversity was found among populations. Of this 5%, 1.7% was due to allele frequency differences between the two regions and 3.3% was found among populations within regions.

The results of our genetic analyses do not support the 'limited gene flow' hypothesis. Values of Nm below 1.0 indicate that genetic drift is the predominant evolutionary force shaping genetic structure, while values of Nm greater than 4.0 indicate that gene flow is more important (Wright, 1951). Estimates of Nm calculated from the G_{ST} values reported above produced values of $Nm = 3.78$ over all the populations and $Nm = 4.52$ and 4.17 within Sonora and Baja, respectively. We therefore conclude that gene flow among these populations is high enough to prevent the loss of genetic diversity due to genetic drift. These relatively high estimates of gene flow are almost certainly due to the long foraging flights (see below) and migratory behaviour of *Leptonycteris* bats (Wilkinson and Fleming, 1996). Our overall conclusion from the allozyme analyses is that the

Table 2. Levels of genetic diversity in populations of *Pachycereus pringlei*

Population	*n*	*P*(%)	*AP*	H_e
Sonora				
4	48	70.8	2.53	0.217
8	48	66.7	2.44	0.186
2	*48*	*62.5*	*2.33*	*0.181*
Mean/pop.	48	66.7	2.44	0.194
Overall Sonora	144	75.0	2.50	0.199
Baja				
15	48	79.2	2.37	0.191
20	44	66.7	2.63	0.222
16	32	66.7	2.88	0.222
19	27	58.3	2.43	0.209
14	26	58.3	2.36	0.182
10	*15*	*62.5*	*2.47*	*0.216*
Mean/pop.	32	65.3	2.52	0.207
Overall Baja	192	83.3	2.85	0.217
Overall species	336	91.7	3.00	0.215

[a] *n* = sample size; *P* = proportion of polymorphic loci; *AP* = number of alleles per polymorphic locus; H_e = genetic diversity. Population designations are given in Table 1 and Fig. 1.

'limited gene flow' hypothesis cannot explain the geographic distribution of the different breeding systems in *P. pringlei*.

The 'nocturnal pollinator abundance' hypothesis

Female sterility alleles cannot invade populations of self-fertilizing hermaphrodites as easily as male sterility alleles (Charlesworth and Charlesworth, 1978), and sexual selection theory (e.g. Willson, 1979) indicates that males should be more strongly affected by pollinator limitation than females. We thus expect the frequency of males to be especially sensitive to the abundance and flower visitation rates of the major pollinator, *Leptonycteris* bats. This hypothesis predicts that *P. pringlei* should have a trioecious (or dioecious) breeding system in areas of high bat density (e.g. near bat roosts) and that gynodioecious (or hermaphroditic) breeding systems should prevail in areas of low bat density.

We tested this hypothesis in three ways. First, we located the major roosts of *L. curasoae* in Sonora and Baja California during our sex ratio surveys and from museum records and published accounts (Woloszyn and Woloszyn, 1982; Cockrum, 1991). Secondly, to determine whether trioecious populations were associated with high densities of diurnal, rather than nocturnal, pollinators, we documented the visitation rates of birds to flowering cardon plants along a 1 km transect for 2 h beginning at dawn on one morning at four gynodioecious sites (sites 1, 2, 19 and 20 in Fig. 1) and four trioecious sites (sites 4, 8, 12 and 16 in Fig. 1). Bird visitation rates to cardon flowers are highest during this time period (Fleming *et al.*, 1996). Thirdly, to determine the probability that a cardon flower would be visited at least once by a bat, we placed a 1.75 cm^2 piece of paper inside the corolla and perpendicular to its long axis in a total of 9–20 freshly opened flowers on 6–11 plants on one night at the same eight sites. We scored each flower as 'visited' (paper obviously disturbed) or 'not visited' (paper intact) before dawn the next morning. We also noted the time of arrival of the first *Leptonycteris* bat at these sites.

Our results indicate that, as predicted by the 'nocturnal pollinator' hypothesis, trioecious sites were located close to known maternity roosts of the gregariously roosting *L. curasoae* (Fig. 1). In Sonora, a major roost containing thousands of bats occurs on Isla Tiburon. Radiotracking studies have shown that many bats fly 25–30 km or more from this roost to the mainland to feed at cactus flowers (Sahley *et al.*, 1993 and unpublished). Cardon populations within flight distance of this roost contained substantial frequencies of males (Table 1), whereas populations south of this roost lacked males. Similarly, in Baja California, all known *Leptonycteris* maternity roosts are located south of 28°N, as are trioecious populations of cardon. One such roost (near site 11) contained 30,000 females and their young in April 1993. In all, 9 of the 13 trioecious sites we sampled (69.2%) were located within 50 km of a known *Leptonycteris* roost, whereas none of seven gynodioecious sites were within 50 km of a known roost ($P = 0.0043$, one-tailed Fisher's exact test).

Our censuses of bird and bat activity indicated that bird visitation rates to flowering plants were very low and that the probability of a flower being visited at least once per night by a bat was high at all sites. The number of nectar-feeding bird visits per plant per hour averaged 0.29 (range 0.06–0.92) and 0.22 (range 0.05–0.62) at gynodioecious and trioecious sites, respectively. Most of these visits were to single flowers, and the proportion of flowers visited by birds at five sites averaged 0.13 (range 0.03–0.30); we failed to note the number of flowers we watched at the other three sites. Gila woodpeckers (*Centurus uropygialis*) were the most common visitors to cardon flowers at most sites and accounted for 48.1% ($n = 54$) of total flower visits.

The proportion of flowers visited at least once by bats averaged 0.95 (range 0.80–1.0) and 0.88 (range 0.75–1.0) at gynodioecious and trioecious sites, respectively. Trioecious sites differed from gynodioecious sites, however, in that bats arrived before 21.00 h at three of the four trioecious sites but arrived after 21.00 h at three of the four gynodioecious sites. These differences are consistent with the observation that trioecious sites are located closer to bat roosts than are gynodioecious sites. We might expect flowers at trioecious sites to receive more visits per night than those at gynodioecious sites. Our work at Bahia Kino supports this prediction. The number of visits per flower decreased rapidly with increasing distance from a *Leptonycteris* bat roost; the proportion of hermaphrodites was twice as high (0.49 *vs* 0.25) 22 km from the roost compared with 7 km from the roost (Fleming *et al.*, 1994). Conclusive proof that bat abundance is higher at trioecious sites than at gynodioecious sites requires further study. Particularly informative would be the number of pollen grains deposited on cardon stigmas at night. We predict that this number will be higher at trioecious sites than at gynodioecious sites.

A model for the effect of pollinator abundance on the evolution of trioecy

Our field observations support the hypothesis that bat distributions and abundances have influenced the evolution of the breeding system of *P. pringlei*. To further explore the potential importance of pollinator abundance on the evolution of trioecy in cardon, we have developed a phenotypic model of the evolution of trioecy under pollen limitation (Maurice and Fleming, 1995). A key feature of our model is the probability that a flower receives enough pollinator visits to produce outcrossed seeds compared to what is needed to produce selfed seeds. This probability will be a function of the frequency of females in a population. Calculations based on this model show that the main effect of pollen limitation in a species in which hermaphrodites are strongly self-fertilizing is to reduce the fitness of unisexuals relative to hermaphrodites. Our calculations also show that under pollen limitation, trioecy can be a stable reproductive system for a set of values of α , β (Fig. 3). For a given set of relative fertilities (α, β), the stable reproductive system can shift from dioecy to trioecy and from trioecy to gynodioecy when pollen limitation increases as the abundance of pollinators decreases.

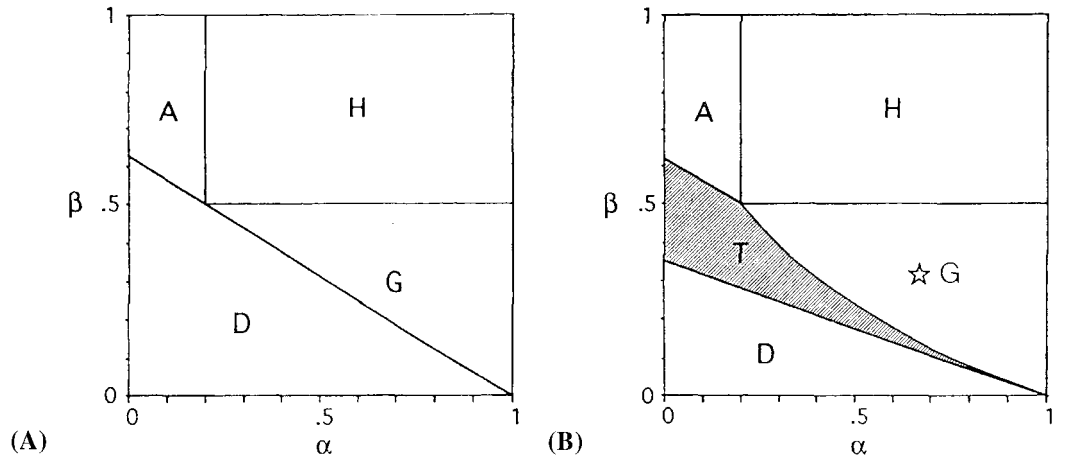

Figure 3. Stable reproductive systems in *Pachycereus pringlei* in the absence (A) and presence (B) of pollinator limitation based on the fertilities of hermaphrodites in terms of their male (α) and female (β) function relative to the fertilities of male and female plants (see Maurice and Fleming, 1995). In both panels, the selfing rate (s) of hermaphrodites is 0.6, and there is no inbreeding depression ($d = 0$). A = androdioecy; H = hermaphroditism; G = gynodioecy; T = trioecy; D = dioecy. For case (B), we have used the model of pollinator limitation of Lloyd (1974), in which $p_f = 1 - f^x$ and x is the average number of pollinator visits a flower receives. Here, $x = 1$, which is compatible with our observations. In (B), the star represents the relative fertilities of hermaphrodites, α and β, measured at Bahia Kino (site 4 in Fig. 1). β, the relative female fertility of hermaphrodites in the absence of pollen limitation, was obtained by measuring fruit set after hand-pollination (Fleming *et al.*, 1994).

Discussion

We have shown that the breeding system of the Sonoran Desert cactus *Pachycereus pringlei* varies geographically. Trioecy is geographically widespread but is not the only breeding system in this species. Males are absent in the southern part of its range in Sonora and in the northern part of its range in Baja California; the breeding system is gynodioecy in these areas.

Gynodioecy could prevail in some areas and trioecy in others for historical reasons owing to limited gene flow between populations. The results of our genetic survey, however, do not support this hypothesis. In fact, the degree of heterogeneity in allele frequencies observed for *P. pringlei* is more similar to values observed for wind-pollinated trees than it is for animal-pollinated, mixed-mating species (Hamrick and Godt, 1989). *Leptonycteris* bats are strong, wide-ranging fliers and clearly have the potential to readily move genes among populations. Genetic analysis of populations of this bat, for example, indicate that bats must occasionally fly between Baja California and the coastal parts of Sonora and Jalisco (Wilkinson and Fleming, 1996). If they make these flights in April and May when cardon is flowering, we might expect little genetic differentiation between Baja and Sonora populations, which is what we have observed. These bats also feed on the fruit of cardon and thus also have the potential to disperse seeds widely.

Geographic variation in climate or soil conditions could also cause geographic variation in the breeding system of cardon. We have not examined the effect of these factors rigorously in this study. Because of the opposite geographic patterns in Baja California and Sonora, however, we suggest that climatic effects on the survivorship of male plants, whose presence in populations varies geographically, are not involved in these patterns, although detailed studies are needed to test this suggestion.

Instead of being related to limited gene flow or geographic variation in climate or physical conditions, our analyses suggest that geographic variation in the breeding system of *P. pringlei*

most probably results from geographic variation in the abundance of *Leptonycteris* bats. Trioecious populations generally occur within 50 km of maternity roosts, which often contain tens of thousands of bats. Gynodioecious populations occur beyond 50 km from these roosts where flower visitation rates by bats presumably are low. Birds are effective but minor pollinators of cardon (Fleming *et al*., 1996); they appear to visit cardon flowers at low rates and frequencies throughout its range. Furthermore, some birds, particularly Gila woodpeckers, are likely to forage over shorter distances than are bats because of their strongly territorial behaviour in the spring (T.H. Fleming, personal observations). Thus, they are unlikely to be a major factor in the evolution of cardon's breeding system.

The hypothesis that trioecy is associated with the abundance of nocturnal pollinators is further strengthened by our theoretical calculations. These show that maintenance of the three sexual phenotypes is possible under pollen limitation and that an increase in this limitation can lead to the disappearance of males. In cardon, males are at a competitive disadvantage to hermaphrodites when pollinators are scarce for two reasons. First, like all plants in the population, they will receive fewer pollinator visits per flower and per plant and hence will export less pollen to flowers of females and hermaphrodites. Secondly, hermaphrodites are likely to have relatively higher selfing rates under pollinator scarcity and hence fewer of their ovules will be available for fertilization by male pollen. Pollinator scarcity will also reduce the fitness of females relative to hermaphrodites but not as strongly as it affects males, especially if high selfing rates in hermaphrodites cause their seeds to have lower fitness than those of the out-crossed seeds of females.

In conclusion, geographic variation in the breeding system of *P. pringlei* appears to be associated with the distribution of *Leptonycteris* bats, and our pollen limitation model provides considerable insight into the geographic patterns. We do not claim, however, that trioecy is stable in the Bahia Kino population of *P. pringlei* because our current estimates of the relative fitnesses of males and females, which are based on only 2 years of observations, are too low to result in stable trioecy (Fig. 3). In particular, male fertility needs to be higher for trioecy to be a stable reproductive system. Because the relative fitnesses of different sex classes are likely to vary from year to year, however, long-term observations are needed to determine the actual fitness of males and females relative to that of hermaphrodites. Whether or not trioecy is stable in some populations of *P. pringlei*, our results suggest that variation in pollinator abundance can lead to substantial differences in breeding systems among plant populations.

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Evolution of reproductive systems in the genus *Silene*

CHRISTINE DESFEUX[1], SANDRINE MAURICE[2], JEAN-PIERRE HENRY[1], BERNARD LEJEUNE[3] AND PIERRE-HENRI GOUYON[1]

[1] *Laboratoire Evolution et Systématique, Université Paris-Sud, Bâtiment 362, F-91405 ORSAY Cedex, France*
[2] *Institut des Sciences de l'Evolution, Place Eugène Bataillon, Université Montpellier II, F-34095 MONTPELLIER Cedex 05, France*
[3] *Institut de Biotechnologie des Plantes, Université Paris-Sud, Bâtiment 630, F-91405 ORSAY Cedex, France*

SUMMARY

The genus *Silene* contains both hermaphrodite, gynodioecious and dioecious species, dioecy being represented in three sections of the genus. To locate the events of change of reproductive systems, we compared ITS sequences of 22 species of *Silene* chosen throughout the whole genus, and four putative outgroup species. Gynodioecy, which is the most common reproductive system within the genus *Silene* and in closely related genera such as *Saponaria* and *Dianthus*, is proposed to be ancestral in the genus. Dioecy has evolved at least twice: once in the section containing *S. latifolia*, and once in the clade containing *S. otites* and *S. acaulis* ssp. *bryoides*. Evolution towards hermaphroditism, associated with evolution of selfing, has also occurred at least twice, in *S. gallica* and *S. conica*.

1. INTRODUCTION

The evolution of dioecy through gynodioecy has received much theoretical attention, concerning both the pathways that lead from hermaphroditism towards dioecy (Bawa 1980; Ross 1982), and the conditions and selective pressures necessary for reproductive systems to change (see, for example, Charlesworth & Charlesworth 1978; Maurice *et al.* 1993; Schultz 1995). Field studies on closely related species with different reproductive systems have suggested that gynodioecy may be an intermediate stage along the pathway from hermaphroditism to dioecy (see, for example, Arroyo & Raven 1975; Lloyd 1976; Webb 1979; Delph 1990; Weller *et al.* 1990). Phylogenetic studies have recently proven to be accurate tools with which to test evolutionary pathways between reproductive systems (Hart 1985; Donoghue 1989; Sytsma *et al.* 1991; Rieseberg *et al.* 1992; but see Swensen & Mullin 1994; Graham & Barrett 1995; Weller *et al.* 1995). They help to determine how many times a particular reproductive system has evolved within a group, and can also reveal reversion events.

The genus *Silene* L. (including *Lychnis* and *Melandrium*) contains several hundred species throughout the world and exhibits a great variety of ecological and morphological characters (Chater & Walters 1964). One of its most interesting feature is its great diversity of reproductive systems: it contains dioecy (males and females), gynodioecy (coexistence of females and hermaphrodites), trioecy (coexistence of males, females and hermaphrodites) and hermaphroditism. Gynodioecy is common in this genus (Knuth 1908; and references therein). Dioecy is represented in three sections, in morphologically and ecologically distinct species. This suggests that dioecy could have evolved several times independently in the genus. *Silene latifolia* Poiret (= *Melandrium album* Rohl.) has, in particular, been studied because it contains morphologically distinguishable X/Y sex chromosomes (Westergaard 1958).

The goal of this paper is to discuss how reproductive systems have evolved within *Silene*: phylogenetic reconstruction and systematics of the genus are detailed in another paper (Desfeux & Lejeune 1996). Here we compare the phylogenetic results with information about the reproductive systems of its species. The first questions we expected to answer concern the evolutionary pathways between the different reproductive systems. Which reproductive system can be considered ancestral in the genus? How many times and from which system(s) have dioecy, gynodioecy or hermaphroditism evolved? A phylogenetic approach may also possibly clarify the conditions and selective pressures that promote changes of reproductive systems. Is the pattern of distribution of the reproductive systems informative about the possible mechanisms that have been involved (or that cannot have been involved) in the evolution of reproductive systems in the genus? If there are multiple origins of certain reproductive systems, are these events confined to a particular region of the phylogenetic tree? Is there a relationship between the ecology of the species (altitude, dryness) and their reproductive systems? What are the relationships between dioecious species and the gynodioecious species that can be expected to have a nucleo-cytoplasmic determination of sex (see, for example, *S. vulgaris*, Marsden-Jones & Turrill 1957; B. Charlesworth, personal communication)?

Because of the large size of the genus, we reconstructed the phylogeny using only a sample of species of the genus: we have investigated the relationships

among 13 out of the 44 sections of the genus *Silene*, represented by 22 species. Priority was given to the sections containing widespread species and species for which the reproductive system is known (table 1). As distant outgroup species, we used two members of the Caryophyllaceae, sub-family Silenoideae: *Dianthus seguieri* and *Saponaria ocymoides*. A total of two other Silenoideae were also taken as putative outgroup species: *Agrostemma githago* and *Cucubalus baccifer*. To reconstruct the phylogeny, we compared the sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (Baldwin 1992; Baldwin *et al.* 1995).

Sequences have been registered at EMBL under accession numbers U30945-U30997 and U32566-U32569. Results of the phylogenetic reconstruction are summarized in figure 1.

2. DESCRIPTION OF THE REPRODUCTIVE SYSTEMS IN *SILENE*

Table 1 summarizes our current knowledge concerning reproductive systems in the genus *Silene*. Some records came from the literature, while others are derived from personal observations of plants in the field or in the greenhouse. The precise statement of the reproductive status of a given species is often difficult. In *Silene*, many species have been reported to contain hermaphrodite individuals and individuals that produce female flowers. These latter individuals can be either purely female or gynomonoecious i.e. producing both female and hermaphrodite flowers on a single plant. The presence of gynomonoecious individuals as well as hermaphrodite individuals and female individuals is quite common in gynodioecious species (Baker 1948; Pomonarev & Demyanova 1975; Koelewijn 1993). In that case, the term gynodioecy is not completely accurate and thus we will also use here gynodioecy–gynomonoecy (table 1) to describe this breeding system. The term gynomonoecy has been used to describe monomorphic populations in which every individual produces both female and hermaphrodite flowers, for example many Compositae (Lloyd 1979). Here it will be used in a broader sense to indicate that some individuals bear both female and hermaphrodite flowers. Unfortunately, the literature is not always clear on whether female flowers are borne by pure female or gynomonoecious individuals, and how frequent they are. We have thus considered species in which female flowers have been observed to be gynodioecious–gynomonoecious species. As Lloyd (1976) stated for gynodioecy, gynodioecy–gynomonoecy includes a wide range of conditions. It includes species in which, besides hermaphrodite plants some gynomonoecious individuals with a low proportion of female flowers can be found, whereas female plants are rare (e.g. *Silene noctiflora*). The boundary between this form of gynomonoecy and hermaphroditism is unclear and arbitrary. At the other extreme gynodioecy–gynomonoecy includes species in which gynomonoecious and female plants are common and hermaphrodites plants function mostly as males (e.g. *S. acaulis*, Shykoff 1988). Here again the boundary with subdioecy is arbitrary.

Well-studied gynodioecious *Silene* include: *S. vulgaris*, *S. acaulis*, *S. nutans*, *S. dichotoma* and *S. pendula* (see table 1 for references). Females in these species are common (Marsden-Jones & Turrill 1957; Shykoff 1988; Pettersson 1992) representing more than 50% of the individuals in some populations (Hermanutz & Innes 1994; J. A. Shykoff, personal communication). Female plants and andromonoecious plants carrying both hermaphrodite and male flowers have been recorded in *S. viscaria* (= *Viscaria vulgaris*) (Jennersten *et al.* 1988) and in *S. roemeri* (Correns 1928; Chater & Walters 1964). Schutz (1890, cited in Knuth 1908) reported the presence of males and andromonoecious individuals in *S. vulgaris* and *S. nutans*, but this has not been confirmed by other workers. Some species are predominantly self-pollinating, but female flowers or individuals can sometimes occur in these species (*S. coronaria*, *Agrostemma githago*, *S. noctiflora*, see table 1). In *Agrostemma*, different levels of self-pollination, from protandry to automatic self-pollination, have been reported in the literature, in different populations and within the same population (Knuth 1908). Thus, it is largely possible that reproductive systems (sexual types) and breeding systems (level of autogamy) are variable not only within the genus but also within some species, among or within populations.

'True' hermaphrodite *Silene* species are probably not as numerous as floras would suggest. Gynodioecy is usually not noted in floras. Species of *Silene* in which no female flowers have been described (e.g. *S. gallica* and *S. conica*) are highly autogamous (and even cleistogamous) species. At least five sections contain cleistogamous species or species in which some individuals carry cleistogamous flowers (see table 1). Other species from these sections are recorded as hermaphrodite (at least no females have been seen in these sections). Only two representatives of these five sections: *S. gallica* (section Silene) and *S. conica* (section Conomorpha) were included in our phylogenetic analysis.

Dioecy exists in three sections of the genus. In the section Elisanthe (*S. latifolia*, *S. dioica*, *S. diclinis*), in the section Otites (*S. otites* and *S. pseudotites*) and in the section Nanosilene (*S. acaulis* ssp *bryoides*). In *S. acaulis* the subspecies *bryoides* (= ssp. *exscapa*) is dioecious, whereas the other subspecies *longiscapa* and *cenisia* have been described as trioecious (males, females and hermaphrodites), subdioecious (males, females and a few hermaphrodites) or gynodioecious with hermaphrodites exhibiting various degrees of maleness in the French and Swiss Alps (Bock 1976; S. Maurice, unpublished data; J. A. Shykoff, personal communication) and in North America (Shykoff 1988; Hermanutz & Innes 1994).

3. GYNODIOECY AS THE ANCESTRAL REPRODUCTIVE SYSTEM

The evolution of dioecy has been considered a sequence beginning with hermaphroditism, proceeding through gynodioecy, and finally reaching dioecy (see Darwin

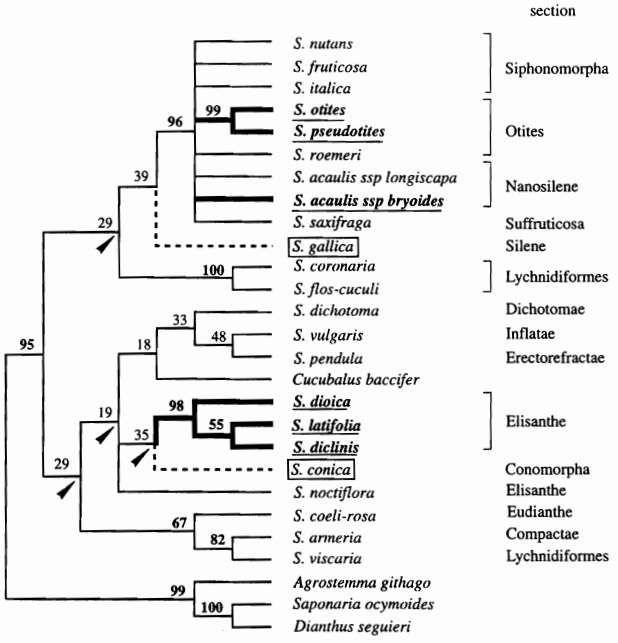

Figure 1. Strict consensus of the 72 most parsimonious trees obtained after a heuristic search with PAUP 3.1.1 (Swofford 1993) (580 steps, CI = 0.478, RI = 0.568, 165 informative sites, uninformative characters excluded, gaps treated as missing data, Desfeux & Lejeune 1996). The numbers on the left of the nodes are bootstrap estimates (in percentage). They indicate the number of times the group consisting of the species which are on the right of the node occured among the trees, based on 1000 bootstap replicates (Felsenstein 1994). Values superior to 50% are indicated in bold. Arrows point out the nodes that collapse in the consensus tree obtained when insertion–deletion events are considered as a fifth base. Note that *Cucubalus* which was initially chosen as an outgroup species falled within the genus *Silene*. Dioecious species are noted in bold style and branches where dioecy has evolved are relatively thick and black. Hermaphrodite species are framed and branches where hermaphroditism has occurred are relatively thick and grey. All the other species (including the outgroup species) can be considered as gynodioecious (see text and table 1).

1877; Charlesworth & Charlesworth 1978; Ross 1982; Delph 1990; Weller *et al.* 1990). The fact that female flowers and individuals have been noted (even if in low frequency) in numerous species of the genus *Silene* (see table 1 and figure 1), and also in numerous species of all the other genera of the sub-family Silenoideae (*Dianthus*, *Saponaria*, *Gypsophila*) and the sub-family Alsinoideae (*Cerastium*, *Minuartia*, *Arenaria*, *Stellaria*) (Knuth 1908; Pomonarev & Demyanova 1975) compromises the hypothesis that hermaphroditism is ancestral in the genus *Silene* and rather supports the idea that gynodioecy is ancestral. Our position differs from that of Weller *et al.* (1995) who postulated a hermaphrodite ancestor for the Hawaian Alsinoideae *Schiedea* based on their assumption of hermaphroditism in the outgroup genera *Minuartia* and *Arenaria*. However, we do not deny that hermaphroditism could be ancestral deeper in the tree.

4. MULTIPLE ORIGIN OF DIOECY

Given this hypothesis of ancestral gynodioecy, it appears clearly in the phylogeny that dioecy has evolved at least twice in the genus (figure 1), once in the section Elisanthe, and at least once in the clade containing *S. acaulis* and *S. otites*. In that clade, species and even sections cannot be distinguished on the basis of their ITS sequences. Anyway, it is noteworthy that dioecy has appeared in two different regions of the tree, and in distal branches of the tree.

All the species that we have included in the phylogeny and that fall within the clade containing *S. acaulis* and *S. otites* are clearly gynodioecious, with non-negligible proportions of females in natural populations. Intermediate stages between gynodioecy and dioecy are present: in *S. otites*, hermaphrodite individuals occur occasionally; in *S. acaulis*, some populations are gynodioecious but hermaphrodites show various degrees of femaleness (some of them even function as pure male) (Shykoff 1988; Hermanutz & Innes 1994; S. Maurice, unpublished data). Such a case of gynodioecy with a gradual reduction of the female fertility in the hermaphrodite has been interpreted as the most common pathway from gynodioecy to subdioecy and dioecy (Darwin 1877; Lloyd 1976; Ross 1982).

In the other region of the phylogenetic tree where dioecy occurs, dioecy is clearer. Hermaphrodites are very rare in *S. dioica* and *S. latifolia*. It is interesting to note that these dioecious species form a well-differentiated clade. The pattern of distribution of the reproductive systems in this region is striking. It shows that gynodioecy can lead either to dioecy or to hermaphroditism: in the close neighbourhood of these dioecious species, are *S. noctiflora*, which is gynodioecious (but with probably a high level of autogamy and few females) and *S. conica*, an autogamous hermaphrodite species. It seems that the evolution towards dioecy was much less gradual in the section Elisanthe than in *S. acaulis* or *S. otites* . The different patterns observed in the two regions of the tree could possibly reflect different selection pressures or conditions leading to dioecy. Alternatively dioecy may simply have arisen longer ago in the section Elisanthe.

The occurrence of dioecy has been correlated with ecological factors (see Bawa 1980; Thomson & Brunet 1990; Renner & Ricklefs 1995). Within *Hebe* (Delph 1990) and within *Schiedea* (Weller *et al.* 1995), in which multiple evolution has been proposed, a strong correlation was found between the distribution of dimorphism and a particular habitat (respectively high altitude and dry habitats) suggesting that ecological factors might have favoured the evolution of dimorphism. Contrasting with these situations, in the genus *Silene* it is unlikely that the same ecological factors were responsible for the evolution of dioecy in all three sections where it occurs. Indeed, the three sections in which dioecy is found are morphologically and ecologically very different. *S. otites* grows in dry habitat and is anemophilous, presenting greenish inconspicuous flowers, characters which are part of the syndrome of dioecy (Bawa 1980). Anemophily is not found in other sections. *S. acaulis* grows at high altitudes or in arctic zones, and forms cushions covered with pink flowers during the flowering season. *S. latifolia* and *S. dioica* are widespread and, unlike *S. otites*, display big

Table 1. *Reproductive systems and life cycles of the species studied*

(Superscript characters refers to the origin of the information. For the life cycles, information mainly came from floras. We included in this table species that have not been included in the phylogeny, but have been described as bearing cleistogamous flowers. The nomenclature for the species is that of Flora Europeae, modified following Med-checklist. For the sections, we have kept the names used in Flora Europeae. The section name Lychnidiformes is adopted for the species that were under the genus name Lychnis in Flora Europeae. H = hermaphroditism, GD = gynodioecy, GM = gynomonoecy, AM = andromonoecy, D = dioecy, SD = subdioecy, T = trioecy, A = annual, B = biennial, P = perennial, S = self-pollination, O = outcrossing, Cl = cleistogamous.)

species (life cycle)	reproductive system	breeding system
subfamily Silenoideae		
Dianthus seguieri (P)	GD[1]	
Saponaria ocymoides (P)	GD[1]	
Agrostemma githago (A)	GD[1]	S[1]
Cucubalus baccifer (P)	GD[1]	
Silene		
sect. Lychnidiformes		
Silene coronaria (P)	GD[1,17]	S[1]
S. flos-cuculi (P)	GD-GM[1,2] H[2,13,15]	O[15]
S. viscaria (P)	GM-GD[1] GD-AM[11]	
sect. Siphonomorpha		
S. italica (P)	GD-GM[17]	
S. nutans (P)	GD-GM[1,7,9,17]	
S. fruticosa (P)		
sect. Otites		
S. otites (B, P)	D, SD[6]	O
S. pseudotites (B, P)	D, SD[6]	O
S. roemeri (B, P)	GD-AM[3,6]	O
sect. Inflatae		
S. vulgaris (P)	GD[4,10,14], T[1]	
sect. Suffruticosae		
S. saxifraga (P)	AM-GM[1], GM[17]	
sect. Nanosilene		
S. acaulis ssp *longiscapa* (P)	T[8]	O
S. acaulis ssp *bryoides* (P)	D[8]	O
S. acaulis (P)	GD-T [12,16]	O
sect. Compactae		
S. armeria (A, B)	GD[1,17]	
sect. Elisanthe		
S. noctiflora (A)	H[1], GM-GD[1,7]	S
S. latifolia ((A)-P)	D[6]	O
S. dioica (P)	D[6]	O
S. diclinis (P)	D[6]	O
sect. Rigidulae		
S. inaperta (A)	H	Cl[1]
sect. Eudianthe		
S. coeli-rosa (A)	GD[17]	
sect. Erectorefractae		
S. pendula (A)	GD[5,17]	
sect. Dichotomae		
S. dichotoma (A)	GD[3]	
sect. Scorpioides		
S. nocturna (A)	H	S,Cl[6]
sect. Silene		
S. gallica (A)	H[1]	S,Cl[1]
S. cerastioides (A)	H[1]	Cl[1,17]
sect. Dipterospermae		
S. apetala (A)	H	Cl[1]
sect. Conomorpha		
S. conica (A)	H	S[1,17]
S. conoidea (A)	H[17]	Cl[17]

[1] Knuth 1908, [2] Blaringhem 1924, [3] Correns 1928, [4] Marsden-Jones & Turill 1957, [5] Heslop-Harrison & Heslop-Harrison 1958 (cited in [11]), [6] Chater & Walters 1964, [7] Pomonarev & Demynova 1975, [8] Bock 1976, [9] De Bilde 1984, [10] Dulberger & Horovitz 1984, [11] Jennersten *et al.* 1988, [12] Shykoff 1988, [13] Biere 1991, [14] Pettersson 1992, [15] Hauser & Loeschcke 1994, [16] Hermanutz & Innes 1994, [17] S. Maurice & C. Desfeux, personal observations.

flowers, either red or white. Studies are still needed in the genus *Silene* to determine the influence of ecological factors on the evolution of dioecy.

5. HERMAPHRODITISM AND CLEISTOGAMY. EVOLUTION OF AUTOGAMY

Two hermaphrodite species were included in this study, *S. gallica* and *S. conica*, from two different sections. They show that hermaphroditism has arisen at least twice from gynodioecy. Such events could be considered as reversions from gynodioecy to hermaphroditism or as evolution towards autogamy. These species are highly autogamous and cleistogamy is present in the two sections that these species represent. Thus cleistogamy also arose twice. At least three other sections, not included in this study, contain cleistogamous species. Thus it is highly probable that cleistogamy has arisen several times independently. One to several reversions from dimorphic breeding systems to hermaphroditism have also been found in *Fuchsia* section *Skinnera* (Sytsma *et al.* 1991) and in *Schiedea* (Weller *et al.* 1995), both groups in which gynodioecy also evolved one to several times towards dioecy or subdioecy. This suggests that in groups where hermaphroditism, gynodioecy and dioecy occur, groups with obviously labile reproductive system evolution, hermaphroditism could be derived rather than ancestral more commonly than it is usually thought. Phylogenetic analyses will be particularly useful in distinguishing these different cases of hermaphroditism. Theoretically, reversions from gynodioecy to hermaphroditism are not surprising because the conditions for the maintenance of females are rather stringent (Charlesworth & Charlesworth 1978; Gouyon 1983; Gouyon *et al.* 1991).

6. SILENE, A GENUS IN WHICH NUCLEOCYTOPLASMIC GYNODIOECY HAS EVOLVED TOWARDS DIOECY?

Recent models developed by Maurice *et al.* (1993) and Schultz (1995) show that gynodioecy in which sex is determined both by nuclear and cytoplasmic genes can evolve towards dioecy and that this is not more difficult than when the sex determination is purely nuclear. Although sex determination in gynodioecious species has often been found to be nucleo-cytoplasmic and dioecy is often present among families with gynodioecy (Maurice *et al.* 1993), there is as yet no clear case in the literature of nucleo-cytoplasmic gynodioecy evolving towards dioecy. In several species of *Silene* some evidence suggests a nucleo-cytoplasmic determination of sex: complex segregations of sexual phenotypes in *S. vulgaris* (Marsden-Jones & Turrill 1957; D. Charlesworth, personal communication), high percentages of females in some populations of *S. acaulis* (Hermanutz & Innes 1994), and female fecundity advantage too low to explain the maintenance of females with nuclear sex determination in *S. vulgaris* (Dulberger & Horovitz 1984; Pettersson 1992) and *S. nutans* (C. Desfeux, unpublished data). This applies both to species that show a tendency towards dioecy and species that do not. The results of our phylogenetic analysis show that those species suspected of nucleo-cytoplasmic sex determination are scattered throughout the phylogenetic tree and some lie close to dioecious species. In addition, an interaction between the Y chromosome and cytoplasmic factors has been suggested as an explanation for sex-ratio distortion in *S. dioica* and *S. latifolia* (= *S. alba*) (Taylor 1994). Altogether, this strongly suggests that dioecy in the genus *Silene* could have evolved from an originally nucleo-cytoplasmic gynodioecy.

7. CONCLUSION

The main points that emerge from our phylogenetic approach are that, within the genus *Silene*, hermaphroditism is unlikely to be the ancestral breeding system, but seems to have been derived at least twice from a gynodioecious ancestral state and is associated with evolution towards autogamy. Dioecy also arose twice, maybe three times, under very different ecological conditions. Future studies should determine the extent of nucleo-cytoplasmic sex determination in gynodioecious species of *Silene* and study the consequences of the evolution of hermaphroditism or dioecy on this nucleo-cytoplasmic interaction.

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Reproductive characters in a gynodioecious species, *Silene italica* (Caryophyllaceae), with attention to the gynomonoecious phenotype

LUCILE LAFUMA and SANDRINE MAURICE*

Institut des Sciences de l'Evolution, Case courrier 065, Université de Montpellier II, 34095 Montpellier cedex 05, France

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Gynodioecious populations (i.e. populations with female and hermaphrodite individuals) often contain a third phenotype with an intermediate sex expression. In *Silene italica*, this phenotype is characterized by a mixture of pistillate and perfect flowers and is thus gynomonoecious. To characterize sexual functions of these gynomonoecious individuals and their potential influence in the maintenance of gynodioecy, reproductive characters of the three sexual phenotypes were compared over 2 years in several families of *S. italica* produced by crossing. We found that gynomonoecious individuals were intermediate for flower production and female fertility characters, although they did not always significantly differ from female individuals. Perfect flowers of gynomonoecious and hermaphrodite plants were similar in size and pollen production. Gynomonoecious individuals were thus intermediate in female and in male functions. Family effects were found for most of the characters. The female advantage (i.e. the fertility of females compared to the female fertility of pollen producing plants) was not dramatically different when gynomonoecious plants were taken into account. *Biological Journal of the Linnean Society*, 2006, **87**, 583–591.

ADDITIONAL WORDS: female advantage – flower production – flower size – fruit set – pollen production – seed weight.

INTRODUCTION

Among plant-breeding systems, gynodioecy, in which both female and hermaphrodite individuals occur within the same species, is represented in approximately 10% of angiosperm species (Delannay, 1978). Because gynodioecy may be a possible intermediate state between hermaphroditism and dioecy (Thomson & Brunet, 1990), this system has been extensively studied, particularly regarding the maintenance of female, or male-sterile, individuals (Lewis, 1941; Lloyd, 1975). Few studies, however, have paid attention to individuals with an intermediate, or partially male-sterile, phenotype (i.e. individuals with a mixture of pistillate and perfect flowers or with mixed flower types), even though it is known that they occur in many species described as gynodioecious (for a review, see Koelewijn & van Damme, 1996; for species of *Silene*, see Desfeux *et al*., 1996; Jürgens, Witt & Gottsberger, 2002). Furthermore, the frequency of partially male-sterile individuals in natural populations is often comparable to that of females (Koelewijn & van Damme, 1996). In studies of this reproductive system, treatment of partially male-sterile individuals is variable. They have sometimes been excluded from the analysis (Shykoff, 1988), included in the sterile category (van Damme & van Delden, 1982), or included in the hermaphrodite category because they produce some pollen (Widen, 1992; Koelewijn & van Damme, 1995a).

The species *Plantago lanceolata, Plantago coronopus*, and *Silene vulgaris*, which all contain partially male-sterile individuals, have a nucleo-cytoplasmic determination of sex (van Damme & van Delden, 1982; van Damme, 1983; Koelewijn & van Damme, 1995a, b; Charlesworth & Laporte, 1998) and the

*Corresponding author. E-mail: maurice@isem.univ-montp2.fr

crossing data available for *S. italica* also support such a determination of sex (L. Lafuma & S. Maurice, unpubl. data). The dynamics of gynodioecy with such an inheritance depends on (1) fertility differences between the cytoplasmic types; (2) the cost of a male-fertility restorer gene in a cytoplasmic type it does not restore; and (3) the magnitude of its advantage in the cytoplasmic type it restores, which depends on the relative female fertility of male-steriles, or females, compared to restored individuals, or hermaphrodites, within this cytoplasmic type (Charlesworth, 1981; Delannay, Gouyon & Valdeyron, 1981). This last parameter, called the female advantage, is usually measured at the population level and not within cytoplasmic type. To the extent that females and hermaphrodites tend to differ in cytoplasmic type in natural populations, the differences between cytoplasmic types and differences between sexes, which are two separate theoretical parameters, will influence the observed female advantage.

In the present study, we focused on the reproductive characters of the three sexual phenotypes of *S. italica* to (1) quantify male and female functions of the partially male-sterile individuals compared to female and hermaphrodite individuals and (2) quantify the influence of their presence on the female advantage. Reproductive characters were compared both at the flower and at the individual level to gain insight into pattern of resource allocation. Because environmental factors may affect reproductive output, we cultivated the plants in an experimental garden. The cytoplasmic type and the genetic background more generally can also influence reproductive characters (de Laguérie *et al*., 1991; Koelewijn, 1996; Gigord *et al*., 1999). We thus chose to compare the fertilities of the different sexual phenotypes within full-sib families because full-sibs have the same cytoplasmic type and share on average half their nuclear genome.

MATERIAL AND METHODS

Plant material

Silene italica (L.) Pers. (Caryophyllaceae) is a short-lived perennial herb commonly found in the garrigue area of southern France. Hermaphrodite individuals bear perfect flowers with two whorls of five stamens. Female individuals bear pistillate flowers with all anthers aborted. Partially male-sterile individuals bear perfect and pistillate flowers; mixed flowers with some, but not all, anthers aborted are rarely seen (one to three mixed flowers seen on less than 5% individuals) so that partially male-sterile individuals can be considered as truly gynomonoecious. Gynomonoecious individuals are common in natural populations of this gynodioecious species (up to 40%; Maurice, 1999).

During the spring of 1998, 40 individuals originating from two populations situated north of Montpellier, Southern France (16 km distant apart from one another; populations E and T in Maurice, 1999) were transplanted in an insect-proof greenhouse at the CEFE (Montpellier) and were hand crossed to obtained full-sib families. Seventeen families were sown at the end of the autumn of 1998, producing from 39 to 96 offspring per family. They were grown in individual pots in an experimental garden (CEFE-Montpellier). Spring 2000 was their first flowering season. Of the 17 families, nine produced enough of the three sexual phenotypes in 2000 to be used in the present study. Sexual phenotypes of the parents and sex ratio in the family progenies are given in Table 1. Families I, K, and P are issued from interpopulation crossing, E, V, and Y from intrapopulation outcrossing and X, Z, and S from selfing (X was included in flower production analyses only because some of its fruits were lost). Most of the plant survived and flowered in 2001. Because of time constraints, only the families with the more numerous gynomonoecious individuals were studied in 2001.

Sex segregations were complex and strongly suggested a nuclear cytoplasmic inheritance of sex because (1) the results could not be interpreted with a simple nuclear determination and (2) differences were obtained between reciprocal crosses.

Sexual phenotypes and data collection

During the spring of 2000 and 2001, respectively, each flower on each individual was tagged and sexed. A proportion of pistillate flowers different from 0 or 1 defines the gynomonoecious phenotype.

Table 1. Origin and sex ratio of the families used in the present study

Family name	Dam	Sire	% F	% G	% H
E	T F	T G	39	11	50
I	E F	T G	38	5	56
K	T G	E G	28	9	63
P	T F	E G	45	7	48
S	E G	E G	55	23	23
V	T G	T H	54	21	26
X	E H	E H	5	48	48
Y	E G	E H	6	23	71
Z	T G	T G	17	15	68

For the parents, the first letter designates the population of origin (E or T) and the second letter designates the sexual phenotype (F for female, G for gynomonoecious, H for hermaphrodite).

Petal sizes of pistillate and perfect flowers produced by the offsprings (in 2000) were measured using a slide calliper. Petal sizes obtained from the parent generation (in 1999) were added for the paired comparison of flower size within gynomonoecious individuals to increase sample size.

In 2000, male fertility was estimated as the number of pollen grains produced per flower (quantity) and as the proportion of full vs. empty pollen grains (quality). For pollen quality, two anthers (one of each whorl) of one perfect flower were put in Alexander stain (Alexander, 1969). Empty pollen grains stain transparent green and are easily distinguishable from full grains (deep pink) using light microscopy. A preliminary study on 14 individuals with two flowers analysed per individual showed less variation in pollen quality within individual than between individuals ($F = 4.07$, $P = 0.0069$); therefore, in the present study, we used only one flower per individual. Pollen quantity was studied with the remaining eight anthers that were digested in 150 μL of sulphuric acid for 24 h to leave nothing but the pollen exine. This was then diluted in 500 μL of 2% Triton solution and precipitated by centrifugation (10 min at 2000 r.p.m). The pellet was washed in 2 mL of ethanol, centrifuged again and dried for 30 min under vacuum to eliminate the ethanol. The pollen was then resuspended in a counting solution (water with 20% glycerine and 20% sucrose) and homogenized for 1 min in an ultrasonic bath (40 MHz). Finally, the pollen was counted on a Malassez haematocytometer cell (the mean of two counts for each flower was used in the statistical analysis). As mentioned previously, mixed flowers with some, but not all, anthers aborted were sometimes observed; these flowers were too few to make a proper analysis and to have a significant effect on individual pollen production, and they were not taken into account in the pollen production analysis.

In 2000 and 2001, we noted the number of fruits produced by 5–10 individuals of each sexual phenotype per family. Fruit set per plant was measured as the ratio of fruits to flowers. However, in 2000, we lacked information on the sex of flowers that yield fruits on gynomonoecious individuals. In 2001, we calculated the fruit set per flower by sex. In 2000, the total seed production of plants was weighed to the nearest 0.001 g (total seed weight). Because harvesting correctly ripen fruits required very frequent observations of plants, we studied only two families, E and V, which were the outcrossed families with the most balanced number of the three sexual phenotypes. The seed weight per fruit was then obtained by dividing the total seed weight by the number of fruits of the plant. In 2001, the seed weights per pistillate or perfect flower fruits for gynomonoecious individuals were obtained. The experimental garden is just across the road of a natural population of *S. italica* and fruit sets obtained by open pollination in the garden were similar to those of natural populations (Maurice, 1999).

Statistical analyses

Some plants, but not all of them, were studied on both years; we thus made separate analyses for 2000 and 2001. For analyses at the plant level, flower number and female fertility components per individual were compared between the sexual phenotypes for families with descendants of all three phenotypes. For analyses at the flower level, we considered the sex of the flower as well as of the sex of the plant and compared pistillate flowers of females and gynomonoecious plants, perfect flowers of hermaphrodites and gynomonoecious plants and both flower sexes within gynomonoecious plants. Because data per flower of each sexual type were not always sufficient for gynomonoecious individuals (2001), data for pistillate and for perfect flowers were analysed on distinct subset of families. Univariate analyses of variance were conducted on individual reproductive traits taking into account sexual phenotype and plant family (this last factor may be seen as a block effect). They were considered as fixed factors. All analyses of variance were performed using the procedure GLM of SAS (SAS, 1989), with type III sums of squares. A Tukey test was performed for comparisons among sexual phenotype means when the sex effect was significant. Wilcoxon's signed rank tests were also performed to compare reproductive characters among different types of flowers (or fruits) within gynomonoecious individuals.

Calculation of female advantage

For comparison with others studies of gynodioecious species, we calculated the classical female advantage (i.e. the fertility of females compared to the female fertility of hermaphrodites). The female advantage was calculated within family to ensure that the calculation is also within cytoplasmic type. The female advantage was first calculated taking into account only hermaphrodites:

$$F/H = \text{mean value of the character for females/mean value of the character for hermaphrodites}$$

and then also taking into account gynomonoecious individuals:

$$F/(H + G) = \text{mean value for females/(mean value for hermaphrodites} \times \% \text{ hermaphrodites} + \text{mean value for gynomonoecious} \times \% \text{ gynomonoecious)}$$

Percentage hermaphrodites and gynomonoecious are within polliniferous types in each family, i.e. %

hermaphrodites = *N* hermaphrodites/(*N* hermaphrodites + *N* gynomonoecious). Data are those obtained in 2000.

RESULTS

Comparison of sexual phenotypes

A family effect was found on all characters but seed weight per fruit (Table 2). The interaction between sexual phenotype and family was never significant.

In 2000, hermaphrodites produced more flowers than females and gynomonoecious plants were intermediate (Tables 2, 3). Plants produced fewer flowers the second flowering season and differences between sexual phenotypes were not significant (Tables 2, 3).

Females had a higher fruit set than gynomonoecious plants in 2000, but there was no significant difference between these morphs in 2001. Gynomonoecious plants had a higher fruit set than hermaphrodites in both years (Tables 2, 3; Fig. 1). In 2000, seed weight per fruit was affected by the sexual phenotype of the plant: fruits of female plants had a higher seed weight on average than those of hermaphrodites, fruits of gynomonoecious being intermediate (Tables 2, 3).

In terms of global production, female plants produced more fruits than hermaphrodites in both years and gynomonoecious individuals were intermediate (Tables 2, 3). The same trend was found for total seed weight but differences between sexual phenotypes were only marginally significant ($P = 0.08$, Tables 2, 3).

Comparison among types of flowers

Because petals were collected and measured before the sexual phenotype of the plant was known, the data are not well distributed and few data are available for

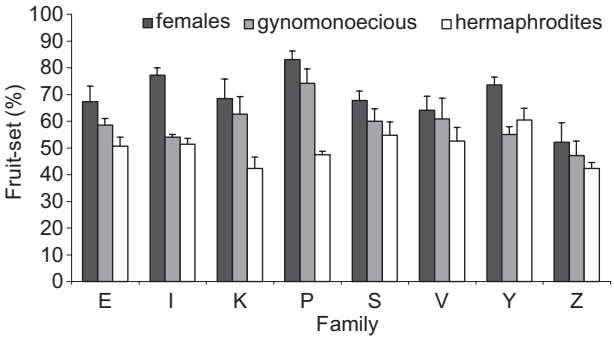

Figure 1. Fruit set per plant by sexual phenotype and family in 2000 (mean ± SE).

Table 2. Analyses of variance of reproductive characters in 2000 and 2001

		2000				2001			
Reproductive character	Source	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
Number of flowers	Sex	2	3421	7.47	**0.0006**	2	380	1.60	0.2042
	Family	8	1642	3.59	**0.0005**	7	782	3.69	**0.0023**
	Sex × Family	16	345	0.75	0.7378	14	148	0.63	0.8436
	Error	549	458			272	237		
Fruit set per plant	Sex	2	0.713	30.13	**0.0001**	2	0.137	3.74	**0.0270**
	Family	7	0.103	4.37	**0.0001**	3	0.447	12.15	**0.0001**
	Sex × Family	14	0.039	1.64	0.0722	6	0.019	0.53	0.7849
	Error	176	0.024			107	0.037		
Seed weight per fruit	Sex	2	160.3	5.19	**0.0097**				
	Family	1	8.9	0.29	0.5944				
	Sex × Family	2	79.4	2.57	0.0884				
	Error	42	30.9						
Number of fruits	Sex	2	458.4	4.09	**0.0184**	2	675.7	9.55	**0.0002**
	Family	7	857.2	7.65	**0.0001**	3	702.1	9.92	**0.0001**
	Sex × Family	14	115.8	1.03	0.4225	6	85.8	1.21	0.3056
	Error	176	112.1			107	70.8		
Total seed weight	Sex	2	190249	2.66	0.0816				
	Family	1	405843	5.68	**0.0218**				
	Sex × Family	2	48419	0.68	0.5133				
	Error	42	71464						

Bold characters indicate significant values ($P < 0.05$). Fruit set per plant variable is Arcsin $\sqrt{}$ transformed.

Table 3. Mean values of reproductive characters

Reproductive character	Phenotype	2000			2001		
		N	Mean	SE	*N*	Mean	SE
Number of flowers	F	199	45.64[a]	1.46	122	36.98	1.55
	G	95	51.69[a,b]	2.02	67	33.39	1.68
	H	282	54.90[b]	1.40	107	30.89	1.51
Fruit set per plant	F	69	68.52[a]	2.09	37	55.08[a]	3.51
	G	51	58.61[b]	2.15	38	54.18[a]	2.76
	H	80	49.74[c]	1.36	44	42.36[b]	2.98
Seed weight per fruit (mg)	F	19	22.86[a]	1.50			
	G	12	18.81[a,b]	1.34			
	H	17	16.76[b]	1.30			
Number of fruits	F	69	31.19[a]	1.60	37	21.97[a]	2.09
	G	51	29.90[a,b]	1.73	38	17.82[a]	1.29
	H	80	26.41[b]	1.09	44	10.93[b]	1.11
Total seed weight (mg)	F	19	617.85	91.93			
	G	12	528.62	43.43			
	H	17	408.53	39.01			

Means followed by different superscripts are significantly different at $P < 0.05$ (Tukey–Kramer test when ANOVA detected a sex effect, fruit set per plant variable is Arcsin $\sqrt{}$ transformed for Tukey-Kramer test). F (and G and H, respectively), female (gynomonoecious and hermaphrodite, respectively) plants; *N*, number of individuals studied for each phenotype.

the size of two types of flowers on the same individual. Perfect flowers, however, were clearly larger than pistillate flowers even within gynomonoecious individuals (Wilcoxon's signed rank test: $N = 9$, $S = 43$, $P = 0.004$). Neither pistillate flower size nor perfect flower size was significantly changed by the sex of the plant that bore the flowers (Tables 4, 5).

Although plant sex did not influence pollen quality (percentage full pollen) of perfect flowers on average, in some families, the perfect flowers on hermaphrodite plants bore the highest pollen quality whereas, in other families, the best pollen was from gynomonoecious plants (Table 4, interaction effect). We found no differences in pollen quantity (number of pollen grains per perfect flower) between hermaphrodite or gynomonoecious individuals or among families (Tables 4, 5).

Fruit set of pistillate and perfect flowers of gynomonoecious individuals did not differ significantly (Wilcoxon's signed rank test: $N = 63$, $S = 790.5$, $P = 0.1365$). The fruit set of pistillate flowers was not influenced by the sex of the plant, nor was that of perfect flowers (Tables 4, 5).

Seed weight of fruits from pistillate and perfect flowers of gynomonoecious plants was similar (Wilcoxon's signed rank test: $N = 24$, $S = 99$, $P = 0.145$). Seed weight of fruits from female plants was only marginally higher than the seed weight of fruits from pistillate flowers of gynomonoecious plants ($P = 0.07$, Tables 4, 5). There was no difference between gynomonoecious plants and hermaphrodites for the seed weight per fruit of perfect flowers (Tables 4, 5).

Female advantage in fertility

We found that females usually showed a fertility advantage, which ranged from 20% to 70% depending on the family and the character considered. Two families showed no female advantage in terms of fruit number (Table 6).

Averaged over families, female advantage in fruit set was 1.39 if gynomonoecious individuals were not taken into account and 1.34 if gynomonoecious individuals were pooled with hermaphrodites. The corresponding values for fruit number were 1.22 and 1.18. The differences between the two types of calculations were thus slight and significant only for fruit set (Wilcoxon's signed rank test: $N = 8$, $S = 34$, $P = 0.025$ for fruit set and $S = 28.5$, $P = 0.148$ for number of fruits).

DISCUSSION

Reproductive characteristics of the gynomonoecious phenotype: at the plant and at the flower level

A clear family effect was demonstrated for many reproductive traits: number of flower, flower size, fruit set, number of fruit, total seed weight, and pollen quality. This implies that, in natural population

Table 4. Analyses of variance of reproductive characters per flower sex

		Female flowers				Perfect flowers			
Reproductive character	Source	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
Size of flowers (2000)	Sex	1	3.48	1.98	0.1776	1	11.23	3.52	0.0652
	Family	1	18.83	10.72	**0.0045**	7	27.69	8.69	**0.0001**
	Sex × Family	1	1.93	1.10	0.3094	7	4.81	1.51	0.1803
	Error	17	1.76			63	3.19		
Pollen quality (2000)	Sex					1	0.078	2.21	0.1419
	Family					7	0.454	12.84	**0.0001**
	Sex × Family					7	0.091	2.56	**0.0219**
	Error					63	0.035		
Pollen quantity (2000)	Sex					1	95.85	0.20	0.6566
	Family					5	384.88	0.81	0.5524
	Sex × Family					5	169.53	0.36	0.8753
	Error					36	476.97		
Fruit set per flower (2001)	Sex	1	0.315	2.88	0.0936	1	0.143	2.33	0.1313
	Family	4	0.440	4.03	**0.0049**	3	0.035	0.57	0.6363
	Sex × Family	4	0.187	1.71	0.1558	3	0.036	0.58	0.6268
	Error	81	0.109			76	0.061		
Seed weight per fruit (2001)	Sex	1	890.5	3.42	0.0694	1	63.2	0.20	0.6576
	Family	4	2308.5	8.88	**0.0001**	3	1333.0	4.18	**0.0090**
	Sex × Family	4	118.3	0.45	0.7685	3	77.2	0.24	0.8667
	Error	57	260.1			67	318.8		

Bold characters indicate significant values ($P < 0.05$). Comparisons have been conducted between female flowers of gynomonoecious and female plants and between perfect flowers of gynomonoecious and hermaphrodite plants. Pollen quality and fruit set per flower variables are Arcsin $\sqrt{}$ transformed.

Table 5. Mean values of several reproductive characters per flower sex

		Female flowers			Perfect flowers		
Reproductive character	Phenotype	*N*	Mean	SE	*N*	Mean	SE
Size of flowers (2000) (mm)	F	16	19.37	0.52			
	G	5	20.37	0.45	32	20.89	0.50
	H				47	21.17	0.309
Pollen quality (2000)	G				31	77.52	4.21
	H				48	81.35	2.93
Pollen quantity (2000) (no. of pollen grains on a Malassez cell)	G				19	77.73	5.35
	H				29	78.06	3.70
Fruit set per flower (2001)	F	42	53.31	3.29			
	G	49	47.86	5.02	41	56.23	3.79
	H				43	45.69	3.04
Seed weight per fruit (2001) (mg)	F	40	51.91	3.18			
	G	27	39.82	3.52	35	52.48	3.18
	H				40	53.04	2.95

F (and G and H, respectively), female (gynomonoecious and hermaphrodite, respectively) plants; *N*, number of individuals studied for each phenotype.

Table 6. Female advantage for fruit set, total number of fruit produced and total weight of seeds produced

	Fruit set		Number of fruits		Weight of seeds	
Family	F/H	F/(H + G)	F/H	F/(H + G)	F/H	F/(H + G)
E	1.33	1.29	1.00	0.96	1.67	1.56
I	1.50	1.49	1.46	1.51		
K	1.62	1.53	1.42	1.31		
P	1.75	1.64	1.32	1.30		
S	1.24	1.18	0.97	0.99		
Y	1.22	1.14	1.17	1.05		
V	1.22	1.24	1.21	1.19	1.28	1.14
Z	1.23	1.20	1.22	1.17		

The female advantage is first calculated taking into account only hermaphrodites then taking also into account gynomonoecious individuals (see methods). F/H, Mean value of the character for females/mean value of the character for hermaphrodites; F/(H + G), mean value for females/(mean value for hermaphrodites × % hermaphrodites + mean value for gynomonoecious × % gynomonoecious).

studies where sex ratios may differ between families, observed reproductive differences between sexual phenotypes may partially be due to family effects. Within families, the main result of the present study is that gynomonoecious plants were intermediate in female and male function between females and hermaphrodites, whether this was linked to plant sex or flower sex depended on the character.

Flower production of sexual phenotypes has often been compared in gynodioecious species (for a review, see Delph, 1996) and hermaphrodites usually produce more flowers than females. This has been interpreted as evidence of selection for increased allocation to pollinator attraction. In the present study, females produced fewer flowers than hermaphrodites and gynomonoecious were intermediate. This is in accordance with sexual selection theory and other empirical studies (Widen, 1992; Koelewijn, 1996), although some authors have found a higher flower production of partially male-sterile individuals (Philipp, 1980; Ågren & Willson, 1991; Guitián & Medrano, 2000).

In many gynodioecious species, pistillate flowers are smaller than perfect ones and three non-exclusive hypotheses have been identified to explain this flower size dimorphism: petals of perfect flowers may be larger than those of pistillate flowers because (1) of an unselected effect of hormones produced by the pollen; (2) they must enclose both ovaries and anthers; or (3) perfect flowers have been selected to be more attractive than pistillate ones (Delph, 1996). In *S. italica*, pistillate flowers were smaller than perfect flowers even within gynomonoecious individuals. The same was also true in *Dianthus sylvestris* (Collin *et al*., 2002) and *Thymus vulgaris* L., a gynodioecious species with rare gynomonoecious individuals (B. Ehlers, pers. observ.). All three hypotheses proposed for gynodioecious species could also explain the dimorphism found within gynomonoecious individuals but this intraindividual polymorphism means that whatever the ultimate cause is, the proximate cause is at least in part pollen production.

Pollen production, in number and quality of grains, was similar between perfect flowers of gynomonoecious and hermaphrodite individuals in *S. italica*. At the plant level, because gynomonoecious individuals produced an intermediate number of flowers and thus evidently an intermediate number of perfect flowers, this result shows that gynomonoecious individuals are also intermediate for male function.

Pistillate flowers may have an increased female function compared to perfect flowers because they do not allocate resources to male function or because their style is more efficient in capturing pollen (Shykoff, 1992). In the present study, female plants had a higher fruit set per plant, higher seed weight per fruit, and, in 2000, produced more fruits than hermaphrodite plants, as is often found in gynodioecious species (for a review, see Couvet *et al*., 1990). Depending on the species and the reproductive character measured, previous studies gave values of partially male-sterile plants between those of females and hermaphrodites or no difference was found between the phenotypes (van Damme, 1984; Ågren & Willson, 1991; Desfeux, 1996; Poot, 1997; Maurice, 1999; Guitián & Medrano, 2000). In one case, the partially male-sterile phenotype had the highest seed production (van Damme, 1984). In the present study, gynomonoecious individuals were intermediate in female function for all significant differences among sexual phenotypes (fruit set per plant, seed weight per fruit, number of fruits) and the same tendency was observed for total seed weight. At the plant level, these results, combined with the intermediate number of flowers, show that female reproductive success of gynomonoecious individuals of *S. italica* was intermediate for all traits studied.

In terms of female function at the flower level, although the female individuals showed a higher fruit set per flower and a higher seed weight per fruit than hermaphrodite individuals, we did not find any significant differences for these characters, either between sexual phenotype within a sex of flower or between sexes of flowers within gynomonoecious individuals. Only fruits of females showed a tendency to be heavier than fruits of pistillate flowers from gynomonoecious plants. Such differences were also not significant in

Silene nutans (Desfeux, 1996) and *Dianthus sylvestris* (Collin *et al*., 2002) but pistillate and perfect flowers within gynomonoecious plants were different for fruit set in natural populations of *S. italica* (Maurice, 1999).

The size and pollen production thus seems to be a property of the type of flower. Female fertility characteristics, whether they are due to stigma properties (Shykoff, 1992) or allocation of resources, are influenced both by flower and individual sexes.

Consequences of the existence of gynomonoecious individuals for the maintenance of gynodioecy

An important parameter for the maintenance and dynamics of gynodioecy is the female advantage (i.e. the relative female fertility of male sterile individuals compared to hermaphrodite, or restored, individuals bearing the same cytoplasmic type). If gynomonoecious individuals share major restorer genes with hermaphrodites, their partially male-sterile phenotype being due to environmental effects or genetic background, they must be grouped with hermaphrodites for the calculation of female advantage in fertility. The calculations in Table 6 show that (1) including gynomonoecious individuals in the calculations diminishes the female advantage but rather slightly and (2) the differences between the female advantage with or without gynomonoecious individuals are smaller than the differences between the families. The risk of mismeasuring this parameter is thus higher if females and hermaphrodites from different genetic backgrounds are compared than if the partially male-sterile individuals are neglected.

It has also been proposed that gynomonoecious individuals could be heterozygous at restorer loci (van Damme, 1983; Koelewijn & van Damme, 1995a) and, with that in mind, Poot (1997) checked whether partially male-sterile individuals somehow outperformed the pure sexes, thus implying overdominance that will help in maintaining polymorphism for restorer/sterility alleles and hence gynodioecy. He concluded that the hypothesis of overdominance could not be invoked in his species because partially male-sterile individuals had a lower female reproductive output than females. However, what must be taken into account in the case of overdominance is the sum of the relative male and female functions of individuals (Gregorius, Ross & Gillet, 1982). Taking the number of perfect flowers as the male fertility of an individual, and the fruit-set or the number of fruits as its female fertility, our data show that a rare gynomonoecious phenotype will not be selected in a hermaphrodite population. This implies that, should the gynomonoecious individuals be heterozygote at restorer genes, their fertility cannot account for the presence of females in population.

In conclusion, our results show that, despite their being common in natural populations, the gynomonoecious individuals do not greatly alter the conditions for the maintenance of females and that, with or without them, the values found for female advantages are between one and two, which is typical of gynodioecious species with a nucleo-cytoplasmic determination of sex (Couvet *et al*., 1990). Nevertheless, it would be interesting to know in detail the genetic determination of sexual phenotype in order to explain the existence of such partially male-sterile individuals.

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Ploidy level and origin of the European invasive weed *Senecio inaequidens* (Asteraceae)

L. Lafuma[1], **K. Balkwill**[2], **E. Imbert**[1], **R. Verlaque**[3], and **S. Maurice**[1]

[1]Institut des Sciences de l'Evolution, Université de Montpellier II, Montpellier, France
[2]University of Witwatersrand, School of Animal Plant & Environmental Science, Wits, Johannesburg, South Africa
[3]Institut Méditerranéen d'Ecologie et de Paléoécologie, Université de Provence, Aix-Marseille I, Centre St Charles, Marseille France

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Abstract. Native to South-Africa, species of the *Senecio inaequidens* complex are presently invasive in Europe, Australia and South-America. Previously, different ploidy levels have been found in these different areas, with only tetraploid individuals reported in Europe, and only diploids in South-Africa and Australia. In the present study chromosome counts and flow cytometry were used to survey DNA ploidy levels in a large sample of 66 native and 21 European invasive populations. One Mexican individual was also added to the study. We found only tetraploid individuals occurring in Europe, whereas both ploidy levels, diploid and tetraploid, were found in South-Africa. Moreover, based on genome size, we suggest that two largely allopatric varieties of diploids exist in South-Africa. The Mexican individual was diploid. We suggest that European tetraploid individuals come from South-Africa and hypothesize that a hybridization event between the two DNA types of diploids occurred in the Lesotho area. The taxonomic difficulties surrounding species of the *S. inaequidens* complex are briefly discussed.

Key words: *Senecio inaequidens*, *Senecio madagascariensis*, invasive species, ploidy levels, flow cytometry.

Polyploidy, defined as the possession of three or more sets of chromosomes, is an important feature of evolution for many plant species. Polyploid plants often possess novel physiological and life-history characteristics not present in their diploid ancestors (Levin 1983, Lumaret 1988). Although Bennett et al. (1998) have shown that polyploidy is more frequent in weeds than in other species, no general relationship between polyploidy and colonizing success has been demonstrated (Barrett and Richardson 1986). However, polyploidy may confer new adaptive attributes (Petit and Thompson 1999), allowing plants to enter new ecological niches, due to (1) a higher genetic diversity (polyploid plants possess more alleles per locus than their diploid ancestors), (2) a higher frequency of heterozygotes (hiding deleterious mutations), and (3) an enhanced gene expression due to gene duplication (Bretagnolle et al. 1998, Barrett and Richardson 1986).

Senecio inaequidens DC. (Asteraceae), a native South African species, has accidentally been introduced with sheep's wool into several

sites in Europe. The first occurrence in Europe was recorded in 1889 in Germany, and other primary invasion sites in Belgium (1922), Scotland (1928), France (1935), Netherlands (1939) and Italy (1947) were documented at the beginning of the twentieth century (see Ernst 1998, Guillerm et al. 1990 for review). The species was firstly restricted to sites of introduction, but then started to spread to several new localities around the seventies (Jovet et al. l975, Dancza and Kiraly 2000, Werner 1994, Ernst 1998) and it is now widespread all over Europe (in Austria and Spain, Polatschek 1984; Switzerland, Mayor 1996; central Europe, Ernst 1998, Dancza and Kirlay 2000; Denmark, H. Pederson, pers. com.; Northern Ireland, P. Hackney, pers.com.). *S. inaequidens* belongs to a complex including *Senecio madagascariensis* and *Senecio harveianus,* a complex which poses extremely difficult taxonomic problems (Hilliard 1977, Leszek 1997, Scott et al. 1998, Radford et al. 2000, K. Balkwill, pers. obs.). Molecular studies have shown that South African *S. madagascariensis* is more closely related to South African *S. inaequidens* than to Madagascan *S. madagascariensis* (Scott et al. 1998, Radford et al. 2000). Different specimens from the National Botanical Institute of Pretoria are classified as *S. inaequidens, S. madagascariensis* and *S. harveianus*. Individuals of the supposed taxa sampled in the localities given by the vouchers do not reveal significant phenotypic differences under controlled growth conditions and cross-fertilizations in the greenhouse between these three so-called species resulted in full seed set giving viable offsprings (Lafuma, unpublished results). Individuals of this complex in Australia, designated as *S. madagascariensis* (Michael 1981), have been accidentally introduced there from South Africa at the beginning of the twentieth century (Scott et al. 1998, Radford et al. 2000) and are now spreading rapidly (Sindel et al. 1998). South American individuals, also classified as *S. madagascariensis,* occur as weeds in agricultural grasslands in the southern part of the Buenos Aires province in Argentina (Verona et al.1982 in Sindel et al.1998) and also appeared recently in Mexico (J. Rzedowski, pers. com.).

Some British and Italian individuals of *S. inaequidens* were examined for chromosome number and were shown to be tetraploid with $2n = 40$ (Harland 1955, Chichiricco 1979). Michael and Radford (Radford et al. 1995) considered that South African individuals, determined by Turner and Lewis (1965) as *S. pellucidus* with $2n = 20$, were in fact two specimens of *S. madagascariensis* complex. In addition, chromosome counts of $2n = 20$ were observed in the invasive Australian weed, *S. madagascariensis* (Radford et al. 1995).

In this study we focussed on the ploidy level of the *S. inaequidens* complex in South Africa and compared it to the ploidy level of *S. inaequidens* in Europe. We test four hypotheses concerning the pattern of distribution of different cytotypes: (1) there are tetraploid and diploid individuals in South Africa and only tetraploids in Europe; (2) diploid and tetraploid cytotypes occur in both continents; (3) only diploid individuals are present in South Africa and both cytotypes occur in Europe; and (4) only diploid individuals are found in South Africa and only tetraploid ones in Europe. Because it is very unlikely that several independent polyploidization events appeared only in Europe, the last hypothesis would be in conflict with the fact that European introductions were supposed to be independent (Ernst 1998). The third hypothesis is for the same reason improbable because tetraploid individuals have already been found in two independent sites of introduction (United Kingdom and Italy). We surveyed species of the complex all over its distribution area in South Africa and in several areas of introduction in Europe. We first established ploidy level by chromosome counts on root tips of three South African individuals used as reference, and then employed flow cytometry, a more rapid and convenient procedure to compare ploidy levels across a wide range of different localities. A Mexican individual was also added to our study to assess its ploidy level in an other area of introduction.

Materials and methods

Plant material. We studied individuals of the *S. inaequidens* complex grown in a greenhouse at Montpellier (France) and originating from various geographic localities of the complex' native range (South Africa) and from Europe and Mexico, areas of introduction (see Table 1 and Table 2 for details).

In South-Africa, seeds were collected from 153 individuals in 66 populations that cover the distribution area of the *S. inaequidens* complex (Table 1). Although Goldblatt and Manning (2000) excluded *S. inaequidens* from the Cape flora, plants collected in the two localities of the Cape area (ACA and ALC populations) did not differ morphologically from those collected in other parts of South Africa (Lafuma, unpublished data). European seeds were collected from 42 individuals in 21 populations, representing at least four sites of introduction in Europe (France, Italy, Belgium and Germany, Table 2). Among these individuals particular attention was paid to two sites of introduction, Mazamet and Liège, were several populations were surveyed (14 individuals in seven populations and 18 individuals in seven populations respectively). In Mexico only one individual from a single population was tested (Table 2). Taxonomic identities for the South African specimens that come from localities documented by the National Botanical Institute of Pretoria or for which Hilliard (1977) gave discriminatory characters (in Natal, under 1500m sea level individuals are supposed to belong to *S. madagascariensis*) are reported in Table 1. All individuals were obtained from germinated seeds.

Chromosome counts. Chromosome numbers were established from counts in root tips of three healthy South African individuals. Growing roots were fixed in 3:1 ethanol:glacial acetic acid at room temperature during 15 days and then refrigerated. For coloration, root tips were treated with acetic carmine and iron (III) acetate and were boiled for 5 minutes. After cooling, root tips were placed in acetic acid between a slide and a cover slip and observed with a light microscope. Chromosome counts were established based on observations of cells in metaphase.

Flow cytometry analysis. Samples consisted of 1 to 2 cm^2 of healthy fresh young foliar tissue. Tissues were finely chopped up with a razor blade in a Petri dish, with 0,5 to 1 cm^2 leaf tissue of *Medicago truncatula* cv Jemalong (internal reference standard, see below). After chopping, 500 µL of ice-cold buffer (pH = 8, 10 mM $MgSO_47H_2O$, 50 mM KC1, 5 mM Hepes and 10 mM Triton X-100) was added and homogenized. The suspension was filtered through a 50 µm nylon mesh, and about 300 µL of nuclei suspension was stained by adding 50 µL of propidium iodide (1mg/mL). The solution was gently mixed, and left at room temperature for about 2 minutes before being analyzed with the flow cytometer (a staining time from 10 seconds to 30 minutes has been tested). We used a consistent methodology for all samples and the repeatability of measurements of the same individuals allows for comparison: we have checked that, within an individual, measures were consistent-both between different dates and between different parts of the plant.

The flow cytometer was a FASScan, working with the software Cellquest (Becton Dickinson, Mountain View, CA). From laser excitation of propidium iodide at 488 nm, we measured reflection at 585 nm to read 2C nuclei DNA contents of 3000 nuclei per sample. We selected *Medicago truncatula* cv Jemalong as an internal reference standard (2C = 0,95; Bennett and Leitch 1995), because preliminary trials have shown that 2C peaks for this species were close to those of diploid and tetraploid *S. inaequidens,* without any overlap, as recommended by Johnston et al. (1999).

Most of the cells are in the G_0/G_1 phase of the cell cycle (G_0 cells are not actively growing or dividing/G_1 cells are actively growing but not dividing) and have a 2n DNA content. The Flow cytometry analysis of isolated nuclei resulted in histograms of their DNA content compared to that of the standard reference, and represented one peak corresponding to the G_0/G_1 nuclei of *S. inaequidens* and one peak corresponding to the G_0/G_1 nuclei of the internal reference *M. truncatula* (Fig. 1). Nuclear DNA content (in pg) of *S. inaequidens* samples was estimated according to the equation : 2C nuclear content of *S. inaequidens* – (0.95xG_0/G_1 peak mean of *S. inaequidens*)/(G_0/G_1 peak mean of *Medicago truncatula*). Only peaks with low coefficients of variations (< 10%) were retained for further analysis.

Assuming that ploidy level of individuals is proportional to their 2C nuclear DNA content, we deduced ploidy level of each sample from the mean DNA content and the ploidy level of individuals from which chromosome counts were made.

Table 1. Inferred ploidy levels of the *Senecio inaequidens* complex populations in its origin area (South Africa). Sample size refers to individuals from different families. Mean Peak *Senecio*/Peak *Medicago truncatula* +/− standard error and mean DNA content (pg) +/− standard error are given for each ploidy level assumed for each population. Putative taxon names refer to the National Botanical Institute (Pretoria, South-Africa) data-base or relevant discriminatory characters given by Hilliard (1977)

Population	Putative taxon name	Coordinates	altitude [m]	Sample size	Peak_*Senecio*/ Peak_*M. truncatula*	DNA amount [pg]	Number of Chromosomes	Ploidy level
AMT	undefined	28°21.05′ S; 29°17.03′E	1679	3	1.37 ± 0.01	1.30 ± 0.01		2
AKE	undefined	28°18.86′ S; 29°12.52′E	1775	3	1.42 ± 0.01	1.35 ± 0.01		2
ASU	undefined	28°11.21′ S; 29°05.26′E	1671	1	1.40	1.33		2
AAA	undefined	27°01.58′ S; 29°52.74′E	1641	4	1.47 ± 0.01	1.39 ± 0.01		2
AAB	*S. harveianus*	32°32.61′ S; 23°58.38′E	798	2	1.60 ± 0.04	1.52 ± 0.03		2
AAR	undefined	29°01.65′ S; 26°24.29′E	1316	1	1.76	1.67		?
ABL	*S. harveianus*	26°39.79′ S; 28°35.37′E	1629	3	1.55 ± 0.05	1.47 ± 0.05		2
ABE	*S. harveianus*	30°58.41′ S; 27°36.34′E	1789	2	2.64 ± 0.03	2.51 ± 0.03		4
ABF	undefined	28°32′ S; 25°15′E	1250	1	1.54	1.47		2
ABH	*S. inaequidens*/ *S. harveianus*	28°15′ S; 28°19′E	1700	3	1.45 ± 0.01	1.37 ± 0.01		2
ABM	*S. harveianus*	29°25.61′ S; 27°51.08′E	2279	7	2.52 ± 0.05	2.39 ± 0.04		4
ABT	undefined	28°17.91′ S; 28°27.16′E	1703	1	1.44	1.37		2
				1	2.67	2.53		4
ABU	undefined	28°16′ S; 26°08′E	1340	1	1.52	1.44		2
ACP	*S. harveianus*	28°56.81′ S; 29°12.69′E	1427	6	1.50 ± 0.22	1.43 ± 0.21		2
ACA	*S. inaequidens*	33°57′ S; 18°28′E		2	1.58 ± 0.02	1.50 ± 0.02		2
ACL	undefined	30°52.86′ S; 25°03.33′E	1486	2	1.43 ± 0.06	1.36 ± 0.06		2
ACR	undefined	32°38.53′ S; 25°21.09′E	705	2	1.32 ± 0.02	1.25 ± 0.02		2
ADE	undefined	28°40′ S; 25°46′E	1240	1	1.56	1.48		2
ADS	undefined	29°50.40′ S; 30°57.65′E	126	2	1.29 ± 0.02	1.23 ± 0.02	20	2
ADU	*S. madagascariensis*	29°51.70′ S; 30°58.25′E	74	2	1.27 ± 0.01	1.20 ± 0.01		2
ADW	undefined	29°35′ S; 26°40′E	1550	1	1.62	1.54		2
AEH	*S. madagascariensis*	28°54.40′ S; 31°26.95′E	486	5	1.24 ± 0.01	1.18 ± 0.01		2
AEA	undefined	33°09.39′ S; 27°27.09′E	229	2	1.24 ± 0.01	1.18 ± 0.01		2
AEL	undefined	31°09.67′ S; 27°45.54′E	1917	6	2.62 ± 0.03	2.49 ± 0.02		4
AGR	*S. inaequidens*	25°57.69′ S; 30°51.68′E	963	4	1.26 ± 0.02	1.19 ± 0.02		2
AGA	undefined	32°27.10′ S; 24°06.23′E	742	1	1.44	1.37		2
AGE	undefined	28°31′ S; 28°25′E	2050	2	2.67 ± 0.04	2.53 ± 0.04		4
AGG	undefined	28°25′ S; 28°31′E	2150	3	2.62 ± 0.01	2.49 ± 0.01		4
AGH	undefined	33°19.12′ S; 26°31′E	630	3	2.36 ± 0.02	2.25 ± 0.02		4

Table 1 (continued)

AGK	undefined	24°58′ S; 30°49′E	1450	2	1.34 ± 0.01	1.27 ± 0.01		2
AGT	undefined	28°32′ S; 28°39′E	2000	2	1.42 ± 0.03	1.35 ± 0.03		2
AHS	*S. inaequidens*/ *S. harveianus*	28°17.30′ S; 29°05.77′E	1647	1	2.42	2.3		4
				2	1.44 ± 0.01	1.37 ± 0.01	20	2
AJE	undefined	28°48′ S; 28°25′E	2000	2	1.45 ± 0.04	1.38 ± 0.04		2
AKF	*S. inaequidens*/ *S. harveianus*	27°38.51′ S; 25°39.21′E	1274	1	1.43	1.36		2
ALC	*S. inaequidens*	33°56′ S; 18°28′E		2	1.50 ± 0.07	1.42 ± 0.07		2
ALG	*S. harveianus*	30°47.98′ S; 27°12.35′E	1717	1	1.27	1.21		2
				2	2.69 ± 0.07	2.55 ± 0.06		4
ALY	undefined	25°10′ S; 30°01′E	1500	2	1.79 ± 0.01	1.70 ± 0.01		?
AMA	undefined	29°21.24′ S; 27°31.37′E	1605	4	1.44 ± 0.03	1.37 ± 0.03		2
AML	undefined	29°47.76′ S; 27°35.45′E	1842	2	2.55 ± 0.05	2.43 ± 0.05		4
AMM	undefined	27°41′ S; 29°35′E	1450	1	1.57	1.49		2
ANG	*S. madagascariensis*	28°51.72′ S; 31°34.69′E	355	5	1.27 ± 0.01	1.20 ± 0.01		2
AOS	*S. inaequidens*	28°33.25′ S; 29°04.69′E	1736	3	1.28 ± 0.04	1.22 ± 0.04		2
AOW	undefined	33°17.72′ S; 23°30.12′E	856	2	2.63 ± 0.15	2.50 ± 0.14	40	4
APK	*S. inaequidens*	28°53.11′ S; 29°19.06′E	1353	6	1.30 ± 0.02	1.24 ± 0.02		2
APA	undefined	26°55.25′ S; 27°27.58′E	1437	1	1.64	1.56		2
APE	undefined	33°58′ S; 25°37.86′E	0	3	2.33 ± 0.05	2.21 ± 0.05		4
APF	undefined	33°33.97′ S; 26°46.11′E	157	2	2.26 ± 0.02	2.15 ± 0.02		4
APJ	*S. madagascariensis*	31°37′ S; 29°32′E	10	1	1.27	1.20		2
ARS	*S. inaequidens*	25°21.63′ S; 30°44.72′E	545	1	1.25	1.19		2
ASR	*S.harveianus*	29°09.78′ S; 30°07.02′E	1435	1	1.30	1.23		2
ASE	undefined	29°49′ S; 28°03′E	2200	2	2.63 ± 0.00	2.49 ± 0.00		4
AST	undefined	30°31.72′ S; 27°22.25′E	1467	3	2.63 ± 0.01	2.50 ± 0.01		4
ABA	undefined	25°52.94′ S; 31°05.37′E	1511	3	1.27 ± 0.02	1.21 ± 0.02		2
ATH	*S. madagascariensis*	29°43.29′ S; 30°29.60′E	830	1	1.38	1.31		2
AUF	*S. inaequidens*	25°34.36′ S; 31°10.87′E	906	2	1.21 ± 0.00	1.15 ± 0.00		2
AUC	undefined	33°18.58′ S; 26°31.24′E	541	1	2.35	2.24		4
AGV	undefined	27°27.67′ S; 30°14.22′E	1776	2	1.27 ± 0.01	1.20 ± 0.01		2
AVL	undefined	26°46.25′ S; 28°26.51′E	1603	2	1.61 ± 0.04	1.53 ± 0.04		2
AWK	*S. inaequidens*	27°20.32′ S; 30°09.48′E	1770	2	1.37 ± 0.08	1.30 ± 0.08		2

Table 1 (continued)

Population	Putative taxon name	Coordinates	altitude (m)	Sample size	Peak_*Senecio*/ Peak_*M. truncatula*	DNA amount (pg)	Number of Chromosomes	Ploidy level
AWA	undefined	27°42.28′ S; 28°55.93′E	1671	1	1.52	1.45		2
AWP	undefined	33°32.14′ S; 23°56.84′E	560	1	1.40	1.33		2
AXX	undefined	29°23.32′ S; 30°01.25′E	1385	1	1.27	1.20		2
ALS	undefined	28°34.68′ S; 29°02.91′E	1649	5	1.26 ± 0.02	1.20 ± 0.02		2
AZA	undefined	30°15.30′ S; 27°10.84′E	1448	1	2.71	2.58		4

As for South African diploid populations, we observed an important variation for DNA content, we tested whether the distribution was bimodal using the coefficient of bimodality (*b*). This coefficient was calculated using the formula $(m_3^2 + 1)/(m_4 + 3(n-1)^2/(n-2)(n-3))$ where m_3 is the skewness and m_4 is the kurtosis of the distribution of a 'n' sample size. Bimodality is significant if *b* is greater than 0.55 (SAS 1996).

Results

Table 1 and Table 2 present DNA values determined respectively for native and introduced *S. inaequidens*. Based on the ploidy level and DNA content of the three reference individuals (Table 1), we considered plants as diploids or tetraploids when their DNA content ranged from 1.13 to 1.6 pg or from 2.11 to 2.65 pg, respectively. No firm conclusion was possible concerning the ploidy level of three individuals from two South African populations, i.e. AAR and ALY. Although their DNA content was closer to that of diploid populations, it did not fall within the distribution patterns of either diploid or tetraploid populations.

In South-Africa, we found diploid populations, tetraploid populations and populations with both cytotypes (Table 1 and Fig. 2). Repeated measures of DNA content in the same material of diploids at different times show much less difference (2 repeats per individuals, 14 individuals measured repeatedly: mean difference among individual repeats = 0.052 pg and maximum variation between two repeats = 0.1319 pg) than difference between all diploid individuals (maximum difference between two individuals = 0.4396 pg). The South African diploid populations show a bimodal distribution of DNA content (the coefficient of bimodality, $b = 0.58$, is significant), probably consisting of two overlapping normal distributions separated at around 1.26 pg of DNA content (Fig. 3). Chromosome counts on one sample of each of these two kinds of populations confirm that they both contain 20 chromosomes (Table 1). Figure 2 shows that diploid

Table 2. Ploidy level of *Senecio inaequidens* in some areas of introduction (Europe and South America). Mean Peak *Senecio*/Peak *Medicago truncatula* +/− standard error and mean DNA content (pg) +/− standard error are given for each population

Population	Putative taxon name	District	Country	Sample size	Peak_*Senecio*/ Peak_Standard	DNA amount [pg]	Ploidy level
DCO	*S. inaequidens*	Copenhagen	Denmark	1	2.57	2.44	4
FBC	*S. inaequidens*	Mazamet	France	2	2.45 ± 0.10	2.33 ± 0.09	4
FLE	*S. inaequidens*	Mazamet	France	2	2.47 ± 0.01	2.34 ± 0.01	4
FMA	*S. inaequidens*	Mazamet	France	4	2.55 ± 0.01	2.43 ± 0.01	4
FRA	*S. inaequidens*	Mazamet	France	1	2.69	2.55	4
FSU	*S. inaequidens*	Mazamet	France	1	2.44	2.32	4
FUC	*S. inaequidens*	Mazamet	France	1	2.58	2.45	4
FZA	*S. inaequidens*	Mazamet	France	3	2.52 ± 0.03	2.39 ± 0.03	4
FLC	*S. inaequidens*	Narbonne	France	3	2.57 ± 0.05	2.44 ± 0.04	4
GKO	*S. inaequidens*	Köln	Germany	1	2.47	2.34	4
HGY	*S. inaequidens*	Györ	Hungary	1	2.56	2.43	4
IRO	*S. inaequidens*	Roma	Italy	1	2.64	2.51	4
PBE	*S. inaequidens*	Amsterdam	Netherlands	2	2.41 ± 0.01	2.29 ± 0.01	4
PDR	*S. inaequidens*	Liège	Belgium	3	2.43 ± 0.06	2.31 ± 0.05	4
PFL	*S. inaequidens*	Liège	Belgium	1	2.46	2.34	4
PHL	*S. inaequidens*	Liège	Belgium	1	2.55	2.42	4
PJU	*S. inaequidens*	Liège	Belgium	1	2.42	2.3	4
PLI	*S. inaequidens*	Liège	Belgium	4	2.65 ± 0.05	2.52 ± 0.05	4
PPA	*S. inaequidens*	Liège	Belgium	2	2.47 ± 0.00	2.35 ± 0.00	4
PWL	*S. inaequidens*	Liège	Belgium	4	2.62 ± 0.06	2.49 ± 0.06	4
RUI	*S. inaequidens*	Northern-Ireland	United-Kingdom	1	2.51	2.39	4
MAM	*S. madagascariensis*	Queretaro	Mexico	1	1.26	1.2	2

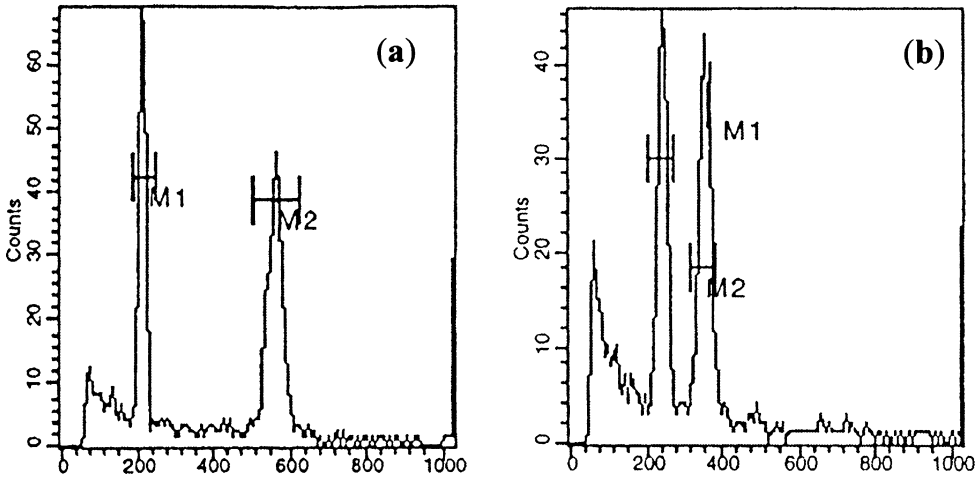

Fig. 1. Histograms of relative DNA contents obtained during the analysis. Left peak refers to internal reference (*Medicago truncatula*), right peak refers to a *S. inaequidens* (s.l.) individual. Histogram of a tetraploid individual (**a**). Histogram of a diploid individual (**b**)

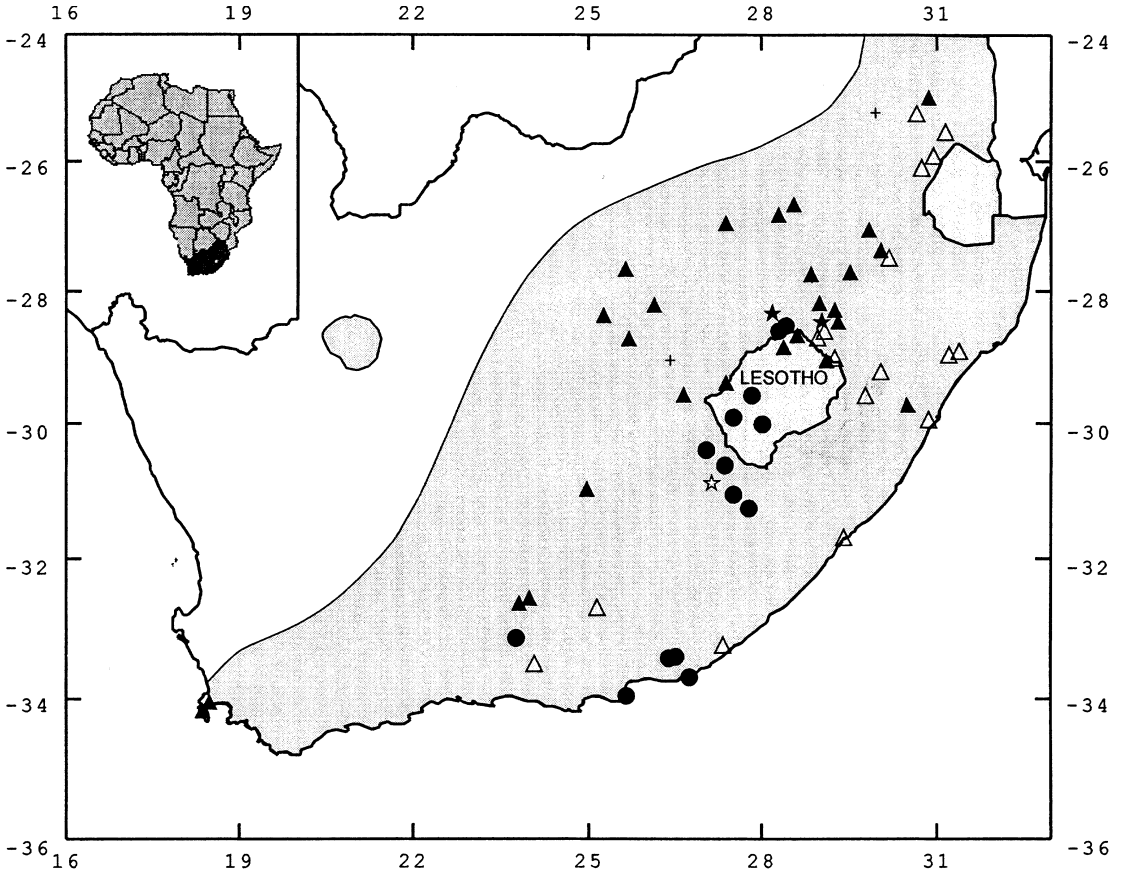

Fig. 2. Cytotype distribution of sampled populations of the *S. inaequidens* species complex in South Africa. ● Indicates tetraploid populations, ▲ and △ refer to diploid populations where ▲ indicates diploid populations with more than 1.26 pg of DNA and △ designates diploid populations with less than 1.26 pg of DNA. ★ and ☆ refer to populations with both cytotypes where ★ indicates that the diploid cytotype contain more than 1.26 pg of DNA and ☆ that the diploids contain less than 1.26 pg of DNA. + denotes populations for which ploidy level was uncertain. Grey area refers to the distribution range of the *S. inaequidens* complex in South Africa

populations with less than 1.26 pg of DNA content are geographically largely isolated from those with more than 1.26 pg of DNA (hereafter referred to as Eastern and Western diploid variety, respectively) and that many tetraploid populations or populations with both cytotypes are found at the border of the two kinds of diploid populations in the Lesotho area. Finally, in South Africa we found no diploid population above 2000 m altitude (Fig. 4).

In Europe all individuals studied were tetraploid (Table 2). There was no relationship between DNA content and the geographic origin of individuals (Fig. 5). In South-America, the only individual studied was diploid and contained less than 1.26 pg of DNA (Table 2).

Discussion

Geographical and ecological origin of the polyploids. Our results show that there are diploid and tetraploid individuals of *S. inaequidens* (s.l.) in South Africa (hypotheses 3 and 4 rejected), but we have found only tetraploid individuals in at least four sites of introduction (France, Italy, Belgium and Germany) in Europe (hypothesis 2 rejected). In particular, we have found no diploid individuals near two documented sites of introduction, Liège in

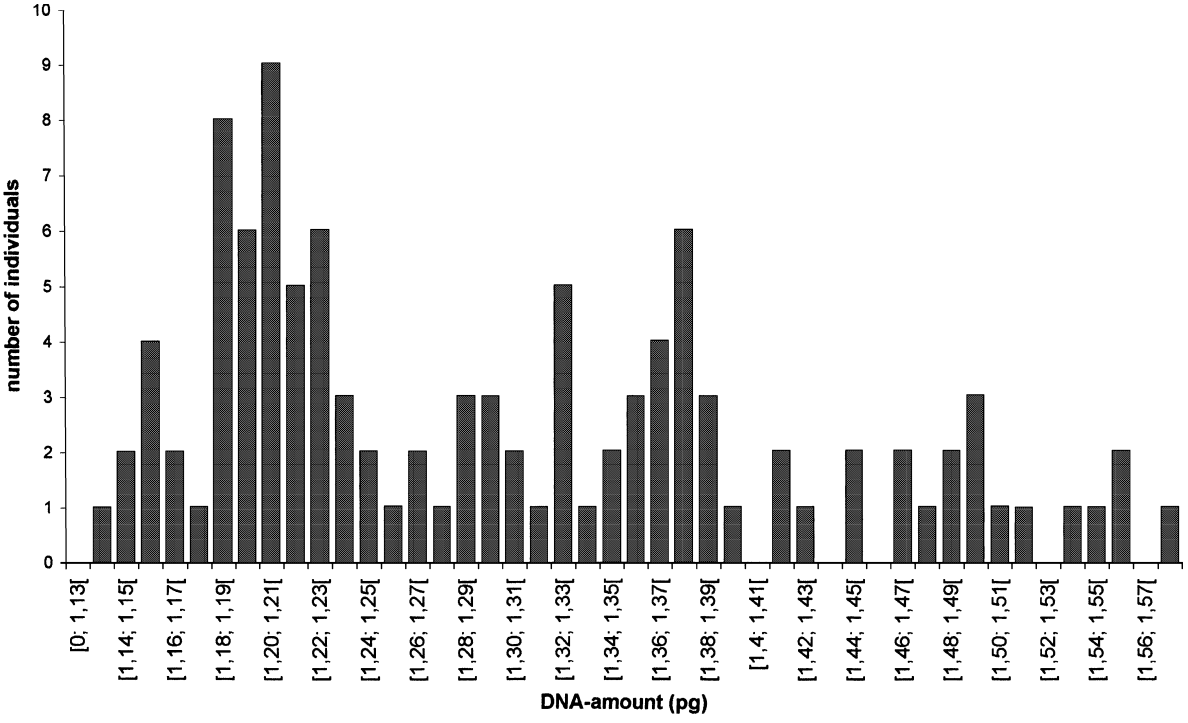

Fig. 3. Number of individuals per genome size class for diploid individuals in South Africa

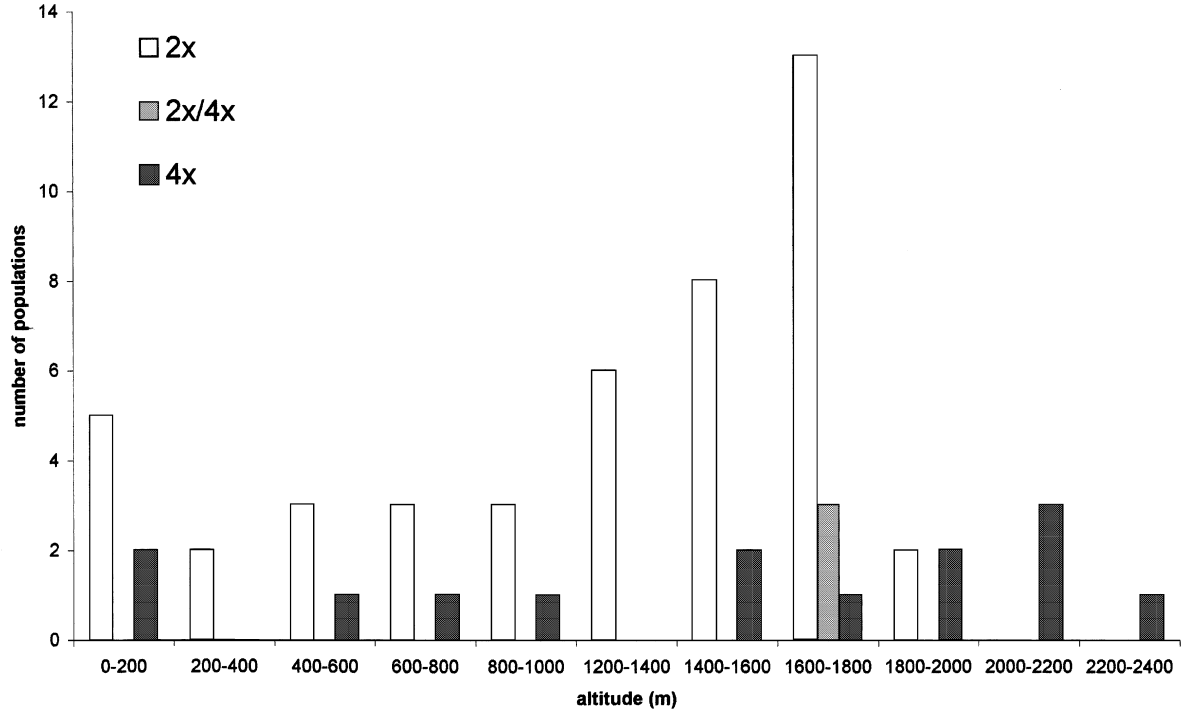

Fig. 4. Occurrence of diploid (2x), tetraploid (4x) and mixed populations at various altitudes in South Africa

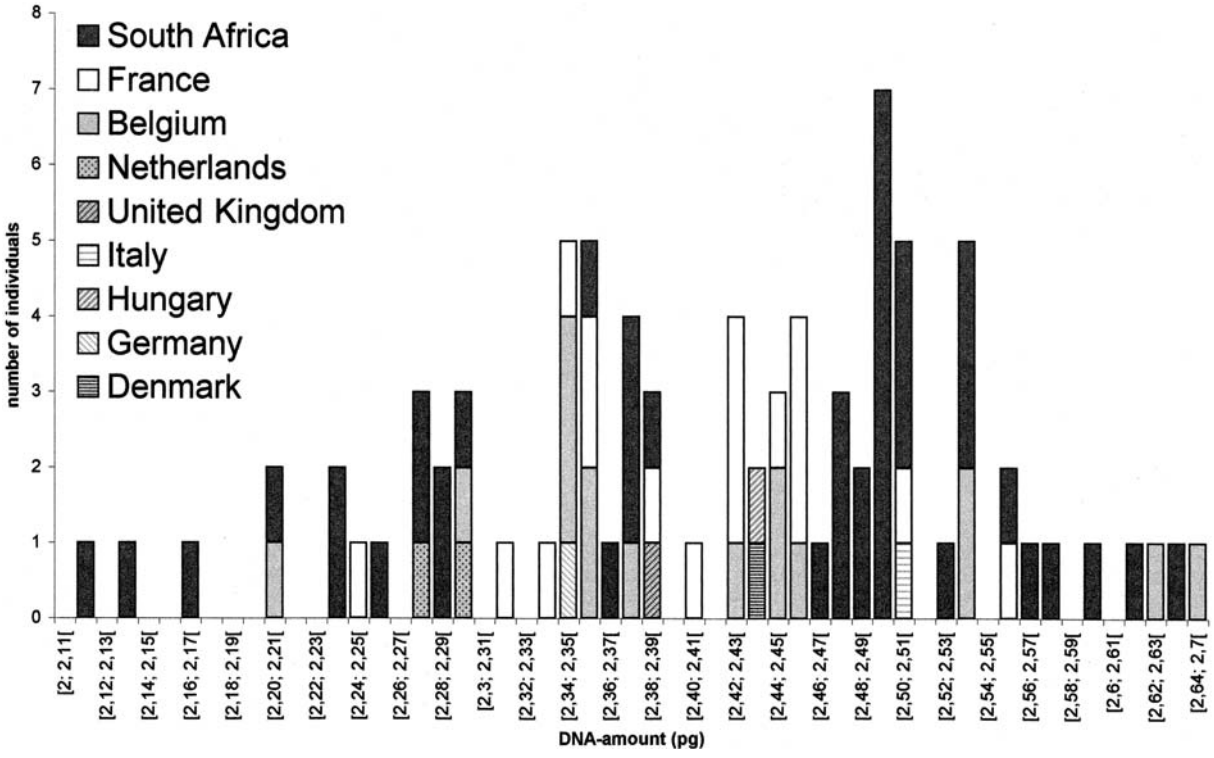

Fig. 5. Number of individuals per genome size class for tetraploid individuals from South Africa, France, Belgium, Netherlands, United Kingdom, Italy, Hungary, Germany and Denmark

Belgium and Mazamet in France (Ernst 1998, Guillerm et al. 1990), even though these areas were extensively sampled. This suggests that only tetraploids were introduced to Europe and does not support the supposition that both diploids and tetraploids were introduced to Europe and that diploids were unable to spread beyond the areas of first introduction. Nevertheless, it is always difficult to demonstrate the non-existence of a phenomenon and here it is not possible to assert that there are (or have been) no diploids in Europe, even though our results do suggest that this is the case. Therefore, we suggest that the first hypothesis may hold true, whereby tetraploidization first occurred in South Africa and only tetraploids were introduced to Europe.

Most tetraploid populations from South Africa occurred in the Lesotho area (see Fig. 2). However, we have also found five other tetraploid populations outside this area (APE, AOW, APF, AUC and AGH) at or in the vicinity of Port Elizabeth. Because Port Elizabeth was an important wool export port at the beginning of the twentieth century, in particular with respect to Europe, we suggest that these populations are secondary, reflecting dispersal of polyploids in South Africa mediated by wool trade from Port Elizabeth. We propose that the polyploidization event happened in the Lesotho area where ecological conditions are probably different, as suggested by altitude. Indeed, tetraploid populations (except the five tetraploid populations from the Port Elizabeth area) were generally found on average at higher altitudes than diploid ones (Fig. 4). All populations containing both cytotypes were found at intermediate altitudes where the distribution of diploid and tetraploid populations overlaps. This may suggest that the geographical patterns of distributions of the two cytotypes reflect differences in their ecological requirements, with polyploids being more capable to tolerate environmental stress.

However, even though higher proportions of polyploids are generally found at higher altitude or latitude than related diploids (Petit and Thompson 1999), there is still debate about the relationship between ecological tolerance and chromosomical valence and no general conclusion could be drawn regarding the ecological distribution of different cytotypes in various polyploid complexes (Thompson and Lumaret 1992).

Little information is available concerning the history of invasion in South America. One chromosome count performed on an Argentinean specimen of *S. madagascariensis* indicates a number of $2n = 20$ (Verona et al. 1982 in Scott et al. 1998). In this study we have shown that the single Mexican individual analyzed was also diploid and that its DNA amount fell into the range of the Eastern diploid variety (Table 2). This suggests that South American populations are not directly related to European ones, but no further conclusion can be drawn regarding a South African or Australian origin.

DNA amount variability. Intraspecific variation in DNA amount remains a hotly debated issue (Bennett and Leitch 2000). Although intraspecific variation in genome size has been detected in various species (for *Rubus alceifolius*, Ansellem et al. 2001; for review see Ohri 1998), many such reports have been refuted because of technical artefacts (Bennett et al. 2000, Ohri 1998). In our study, repeated measures on the same individuals suggest that even though the observed variation may have been overestimated due to methodological errors, intraspecific DNA variation does exist in diploids. The geographical distribution pattern of diploid populations varying in genome size further supports the idea of a bimodal distribution of DNA content. It is recognized that variation in genome size may be correlated with various environmental conditions and adaptive traits (see Ohri 1998). Here, for instance, differences observed in C-values for eastern and western populations seem to be roughly correlated with the distribution of mean annual rainfall in South Africa (http://www.ngo.grida.no/soesa/nsoer/Graphics/national/rain.gif), and this may explain the evolution of two DNA-amount types observed in diploids.

Tetraploid populations and populations with both diploid and tetraploid cytotypes are found at the border of the two kinds of diploid populations in the Lesotho area. Tetraploid populations could have evolved by an autopolyploidization event (simple doubling of a single genome) within one kind of diploid population, or by an allopolyploidization event (hybridization event involving two types of genomes) involving the two closely related DNA-amount types. Because the DNA amount of polyploids is not expected to increase proportionately with ploidy level (genome size in polyploids being biased towards smaller values, Bennett et al. 2000) we could not test which type of polyploidization may have occurred based on the amount of DNA observed among tetraploids. The combination of two facts suggests that the allopolyploidization hypothesis is more parsimonious than the autopolyploidization hypothesis. Firstly, the two different diploid DNA types are geographically separated with tetraploid populations forming a wedge in-between (Fig. 2). Secondly, of the three populations containing both diploid and tetraploid individuals, one contains a diploid individual with low level of DNA, and two contain individuals with high level of DNA content (Table 1 and Fig. 2).

Taxonomic identities of the *S. inaequidens* complex. The distribution of the different taxonomically recognized species of the *S. inaequidens* complex does not coincide with the distribution of genome size. In South Africa, *S. harveianus* and *S. madagascariensis* have distributions included in the distribution of *S. inaequidens*, which cover the distribution of the complex (National Botanical Institute of Pretoria). *S. harveianus* is supposed to occur roughly in the Lesotho area and in the North East of South Africa. Distribution of *S. madagascariensis* is included in the Eastern diploid variety. However, morphological dis-

tinction between species is questionable (K. Balkwill, pers. obs.). Morphological comparisons under controlled conditions and crossing experiments between populations classified as three different species (*S. inaequidens, S. madagascariensis* and *S. harveianus*) were performed with Eastern and Western diploids (with ACP, ANG, APK, ARS and ABL used for morphological comparisons and ACP, ANG and APK used for crossings; Lafuma, unpublished results) and have shown that these populations cannot be distinguished on the basis of taxonomic traits used to classify them. This further suggests that the two kinds of diploids are closely related.

In South America and in Australia, where the weed is diploid, it has been classified as *S. madagascariensis* and the name of *S. inaequidens* was never proposed (Sindel et al. 1998). In Europe, only *S. inaequidens* and *S. harveianus* (Jovet et al. 1975, Tutin et al. 1976) have been proposed to name the species. This suggests that the controversy which has emerged concerning their taxonomy might be related to ploidy differences, often associated with phenotypic differences in the complex (L. Lafuma and S. Maurice, pers. obs.). The existence of different species in South Africa may be a reality, but the degree of differentiation among them has to be further clarified. Genome size in South Africa could be used as a taxonomic character to throw light on this taxonomically confusing complex.

Conclusion

This study has provided new information concerning the ecological and evolutionary origin of the European tetraploid weed *S. inaequidens*. We believe that an allopolyploidization event may have occurred in the Lesotho area between two DNA-amount types of one species of the *S. inaequidens* complex. However, additional work such as molecular studies are needed to confirm our hypotheses. Abbott and Milne (1995) consider this type of information crucial to understand evolutionary changes that might promote the rapid spread of invading species. Difference in ploidy level between Non-European and European invasive plant taxa may be one characteristic that could explain the success of invaders in Europe. Indeed polyploids may be able to adapt more easily to new and variable environments (Levin 1983, Lumaret 1988, Thompson 1991). However, although only tetraploid populations of *S. inaequidens* seem to have been successful in Europe (provided that both diploid and tetraploid populations had been introduced), diploid individuals have colonised Australia and South America. Thus, the relationship between ploidy level and colonizing success in the species needs further study.

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Addresses of the authors: Lucile Lafuma (e-mail: lafuma@isem.univ-montp2.fr), Eric Imbert, Sandrine Maurice, Institut des Sciences de l'Evolution, Université de Montpellier II, Place Eugène Bataillon, 34095 Montpellier cedex 05, France. Kevin Balkwill, University of Witwatersrand, School of Animal Plant & Environmental Science, ZA-2050 Wits, Johannesburg, South Africa. Regine Verlaque, Institut Méditerranéen d'Ecologie et de Paléoécologie, Université de Provence, Aix-Marseille I, Centre St Charles, Place Victor Hugo, 13 331 Marseille cedex 3, France.

Research

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Increase in mate availability without loss of self-incompatibility in the invasive species *Senecio inaequidens* (Asteraceae)

Lucile Lafuma and Sandrine Maurice

L. Lafuma and S. Maurice (maurice@isem.univ-montp2.fr), Inst. des Sciences de l'Evolution – UMR5554, Univ. Montpellier II, FR-34095 Montpellier cedex 05, France.

The evolution of the strength of self-incompatibility in *Senecio inaequidens*, a native of South Africa was investigated in relation to its invasion in Europe. Levels of self-incompatibility were estimated with hand-pollinations in five populations in greenhouse conditions. One population came from the native range of the species and four populations were sampled in Europe from two independent transects of colonization with old and recent populations. Contrary to Baker's law predictions, our results suggest that the species has a sporophytic self-incompatible system maintained in all populations. We suggest that the ability of *S. inaequidens* to colonize new sites with a self-incompatibility system is promoted by its ecological characteristics (perenniality, extended reproductive period, massive seed production, generalist pollinators). In addition, we found that mate availability was increased (1) in the introduced range compared to the native range, (2) in marginal versus central European populations. Possible explanations for this surprising result are discussed.

The breeding system of plants imposes particular constraints on the mating structure and thus eventually on the reproductive success of individuals. One type of breeding system which limits mate availability in plants is self-incompatibility, defined as the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination (de Nettancourt 1977). Self-incompatibility prevents fertilization if pollen and pistil share the same incompatibility type, making selfing but also mating between relatives that have inherited the same incompatibility type impossible. This widespread mechanism thus promotes outbreeding and gene flow in angiosperms and could have played a significant role in their evolutionary success (Hiscock and Tabah 2003). Current phylogenetic and molecular data suggest that self-incompatibility has probably evolved on numerous independent occasions (Steinbachs and Holsinger 2002, Hiscock and Tabah 2003). In parallel, the loss of self-incompatibility has occurred repeatedly in the flowering plants (Richards 1997).

In colonizing species, bottlenecks profoundly affect population size and cause losses of allelic variation at the self-incompatibility locus (or S-locus), reducing both the number of individuals and the percentage of cross-compatible mates. Because self-incompatibility systems require a large number of S-alleles to maintain high levels of cross-compatibility (Byers and Meagher 1992, Vekemans et al. 1998, but see Brennan et al. 2002), self-incompatibility is expected to be a disadvantageous feature in colonizing species. Baker's law (1955, 1967) invokes more generally the importance of reproductive assurance in selecting for self-fertilization in colonizing plants and animals. Evidence for Baker's law has been drawn largely from comparative studies documenting a higher frequency of self-compatible species in islands compared to mainland area or within colonizing species compared to related non-colonizing plant species (Baker 1955, Price and Jain 1981, McMullen 1987). Although these correlations are consistent with a role of reproductive assurance in the colonizing ability, they require further tests by analysing sub-hypotheses since they are usually complicated by correlations between factors that affect the level of cross-pollination (pollinator abundance, mate availability) and those that facilitate selfing for genetic reasons (i.e. by diminishing the inbreeding depression). Small

populations or populations having experienced founder effects are expected to show a reduced rate of inbreeding depression because mildly deleterious alleles can be fixed (Bataillon and Kirkpatrick 2000) and recessive lethal alleles are in lower frequency (Hedrick 2002). These genetic properties thus facilitate the evolution of selfing though Glémin et al. (2001) have shown that quite a strong level of inbreeding depression can be maintained in small populations if deleterious alleles are linked to the S-locus. Besides, even if some self-compatible species have succeeded in colonization compared to self-incompatible species, few studies have clearly demonstrated reproductive assurance as a strong selective pressure for the active dissolution of self-incompatibility system and the evolution of self-fertilization (but see Barrett et al. 1989, Reinartz and Les 1994).

Senecio inaequidens, a member of the family Asteraceae native to South Africa, is currently invading Europe. The progression of *S. inaequidens*, or colonization transect, originating from two different introduction sites, have been documented by botanists in southern France and in Belgium/Netherlands (Guillerm et al. 1990, Ernst 1998). *Senecio inaequidens* is self-incompatible but individuals that showed partial self-fertility have been observed in France (Lopez Garcia and Maillet 2005). We tested if colonization 1) reduced the average compatibility between plants and/or 2) promoted selfing ability by comparing level of self and cross-compatibility in a South African native population compared to a European population and an old population compared to a recent population in each of the two invasion transects.

Material and methods

Study species

The focal species *Senecio inaequidens* is a perennial Asteraceae that frequently invades disturbed habitats such as pastures, vineyards or roadsides. In South Africa, diploid and tetraploid individuals can be found whereas only tetraploid individuals are found in Europe (Lafuma et al. 2003). Several introductions occurred in Europe during the 20th century and well documented historical data are provided for two of them. In Liege (Belgium) the species occurred for the first time in 1922. After 40 years restricted to this site it started to spread and reached Amsterdam (Netherlands) in 1985 (Ernst 1998). In the south of France *S. inaequidens* was introduced in Mazamet in 1936, began to spread in the 70s and was firstly described by botanists near Narbonne in 1983 (Guillerm et al. 1990). Both Liege and Mazamet possessed wool factories and imported sheep wool from South Africa. It is thus supposed that seeds were introduced with the sheep wool and that the two colonization events are independent.

Senecio inaequidens has a highly effective production of fruits (these one-seeded indehiscent fruits will hereafter be named "seeds" for convenience). Some individuals may produce more than 1000 capitula during a flowering period, each capitulum having potentially the capacity to give roughly one hundred seeds (Lopez-Garcia and Maillet 2005). Sexual reproduction starts in spring (April-May), about two months after germination, and may end in late autumn (November-December). Pollination is mostly entomophilous with generalist pollinators. Genetics of self-incompatibility in *S. inaequidens* has not yet been investigated, but self-incompatible Asteraceae usually possess a homomorphic sporophytic self-incompatibility system and so does the congeneric species *S. squalidus* (Hiscock and Tabah 2003).

Sampling

Four populations were sampled in Europe in 2000. We will call "old" the populations at the introduction sites and "young" or "recent" the populations at the end of the invasion transect. Two populations were sampled in France, near Mazamet (43°30′N, 2°24′E – old population) and near Narbonne (43°11′N, 3°00′E – young population). Two populations were sampled along the transect of colonization of Belgium/Netherlands near Liege (50°40′N, 5°39′E – old population) and near Amsterdam (52°12′N, 4°46′E – young population). In 2002, the most likely source area of European populations was found (Lafuma et al. 2003) and a tetraploid South African population, Elliot (31°09′S, 27°45′E), was added to the sample. All sampled populations occurred along roads and had similar densities.

Self-incompatibility in an insect-proof greenhouse

In each population, capitula from regularly spaced plants (ca 3 m apart) were harvested in 2000 or 2002. Plants were numbered and distances between these plants were measured. Progeny of these plants were grown in controlled conditions – in Montpellier, France – until the flowering season. We used one individual from each of ten maternal families for each of the populations harvested. Hand-pollinations were performed in August 2001 for European populations. In April 2003, new hand-pollinations were performed for the European population of Mazamet (the same individuals were used in 2001 and 2003) and for the South African population of Elliot. Hand-pollinations were performed when all the florets of the same capitulum were receptive (the flowering is centripetal

within a capitula but a stigma remains receptive several days if not pollinated). The pollen was collected from the donor plant using a paintbrush, which was then brushed against recipient heads to achieve pollination (Kearns and Inouye 1993). Two treatments were performed on each plant: (1) 3 independent geitonogamous self-fertilization by hand-pollinations, (2) 3 to 5 cross-pollinations each with a different pollen donor of the same population (incomplete diallelic design). Inter-population crosses between the French population of Mazamet and the Belgium population of Liege were also realized: three capitula of each individual of the Mazamet population received pollen from three randomly chosen individuals of the Liege population. After fertilization, we measured for each cross performed the number of viable seeds: these are dark and thick and can be easily distinguished from white, thin, unfertilised ovules. These data were used to calculate the seed/ovule ratio as the number of viable seeds/(viable seeds + unfertilized ovules).

Data analysis

Data from experimental crosses were analysed using general linear model using Proc GLM (SAS 1999). The response variable was the seed/ovule ratio. First, we performed a general mixed-model analysis of variance (ANOVA) considering population (six levels: data from the population of Mazamet realized in 2001 and 2003 were considered as two different levels as environmental factors may affect the self-compatibility system (de Nettancourt 1977)) and cross treatment (two levels: self- versus cross-pollination) as fixed effects. Population was considered as a fixed effect as populations were not chosen at random but because of their position on the invasion transect. The plant effect (nested in population) was specified as random effect. Multiple comparisons of mean values were performed using Tukey test. For each population we also compared the seed/ovule ratio level of cross-pollinations with self-pollinations with a t-test (for paired comparisons). Seed/ovules ratios for cross-pollinations revealed bimodality due to the fact that cross-pollinations are a mixture of compatible and incompatible crosses. Comparison of ratio for self- and cross-pollinations justified that crosses were considered as compatible when their ratio was above 0.1 (Results). A second model was thus used to compare the seed/ovule ratio of cross compatible fertilizations (seed/ovule ratio > 0.1). We compared populations of the same transect of colonization and the population of Mazamet with the South African population independently. The population effect was specified as fixed effect and the plant effect (nested in population) was declared as random effect. For both models:(1) the denominator degrees of freedom were calculated using the Satterthwaite's approximation, (2) type III sums of squares were used to calculate F-ratios and (3) the seed/ovule ratio was transformed using angular transformation in order to satisfy model assumptions (Sokal and Rohlf 1995).

To compare the number of compatible and incompatible crosses between populations, we performed a χ^2 test. Data within and between cells of the 2×2 contingency table are not totally independent as each individual was used in more than one cross. However the variable being tested here is the result of an interaction between two plants and each cross appears only once. Besides we verified that the results were not due to particular individuals by jackknifing the data set over individuals and checking that the data showed the same pattern.

According to self-incompatibility mechanisms, related individuals have a low probability of being cross-compatible. Within a population, there might be a relation between genetic and geographical distance. If such a relation exists in the study populations and if there was a difference in the average geographical distance between plants representing each population, this might affect our comparisons of compatibility levels. We thus performed a t-test on mean distance between parents involved in compatible crosses versus incompatible crosses in each population to exclude the possibility of this bias in our analysis.

Results

Levels of self-compatibility

The seed/ovule ratios obtained by hand-selfing varied from zero to 0.38 (Fig. 1a). The distribution was strongly skewed towards zero: more than 90% of self-pollinations gave less than 0.1 of fertilized seeds and full self-sterility was found for 50% of selfed capitula. The 10 ratios out of the 147 self-pollinations performed that were higher than 0.1 were linked to four specific individuals, each belonging to a different European population (there were at least two capitula with ratio > 0.1 for each of these four individuals). However the seed/ovule ratio for these four selfed individuals was lower than the seed/ovule ratio for cross-pollinations using these same individuals.

The seed/ovule ratios obtained by cross-pollination varied from zero to 1 (Fig. 1b). The ANOVA detected a significant effect of crossing treatment (self-pollination versus cross-pollination, Table 1, Fig. 2). In each population, the self-pollinations differed from the outcrossings (in each population t-test, $p < 0.05$ with Bonferroni correction). The interaction of crossing treatment with population was significant (Table 1) largely because of differences in the seed/ovule ratios in

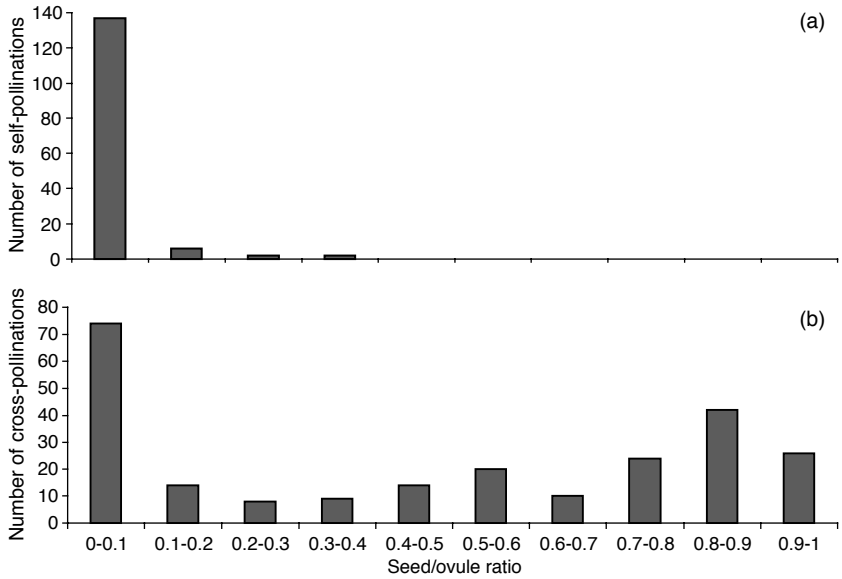

Fig. 1. Distribution of (a) self-pollinations and (b) cross-pollinations in seed/ovule ratio classes over all intra-population crosses.

cross-pollinations between sites. In particular, the seed/ovule ratios of cross-pollinations of Narbonne and Amsterdam were higher than those of Mazamet (2001 and 2003) and Elliot (Tukey test, $p < 0.05$; Fig. 2). No significant differences were found in self-pollinations between sites (Tukey test, $p > 0.05$; Fig. 2). We also found a significant plant effect (Table 1).

Frequencies of compatible crosses

As the majority of selfings gave a seed/ovule ratio below 0.1 and the distribution of seed/ovule ratio of cross-pollinations also showed a mode in the class 0-0.1 (Fig. 1), we considered crosses between plants as incompatible when their seed/ovule ratio was below 0.1. The seed/ovule ratios from compatible crosses were significantly different neither between populations of the same transect of colonization (French transect: $F = 2.46$, $p = 0.133$; Belgium/Dutch transect: $F = 0.13$, $p = 0.718$), nor between the old French population and the South-African population ($F = 3$, $p = 0.108$). This further justified the decision of taking the same threshold of 0.1 in all populations to distinguish incompatible crosses. Among the 64 reciprocal crosses realized, 36 showed reciprocal compatibility, 9 exhibited reciprocal incompatibility and 19 crosses were compatible in one direction but not on the other, i.e. they displayed a reciprocal difference in compatibility. We found more compatible crosses within the old French population than within the South-African population (Table 2a). Intra-population crosses in the recent French population (Narbonne) tended to be more compatible than in the old French population (Mazamet) (Table 2b, marginally insignificant). More intra-population crosses were compatible in the recent Dutch/Belgium population (Amsterdam) than in the old Dutch/Belgium population (Liege) (Table 2b). Crossing within and between the old European populations showed that inter-populations crosses tended to be more compatible than intra-populations crosses (Table 2c). These patterns held under the jackknife procedure over individuals. In each population, there was no difference in mean distance of parents of plant used in cross-pollinations between

Table 1. Mixed model analysis of variance for the seed/ovule ratio from experimental intra-population crosses in *S. inaequidens*. Crosses made two different years (2001 and 2003) on the population of Mazamet were considered as two different populations. The ratio was arcsin($\sqrt{}$) transformed.

	DF	Mean square	F-ratio	p-value
Type of cross	1	25.6638	213.08	**<0.0001**
Population	5	0.7545	3.22	**0.0131**
Type of cross × population	5	0.3641	3.02	**0.0110**
Plant (population)	47	0.2458	2.04	**0.0002**
Residual	329	0.1204		

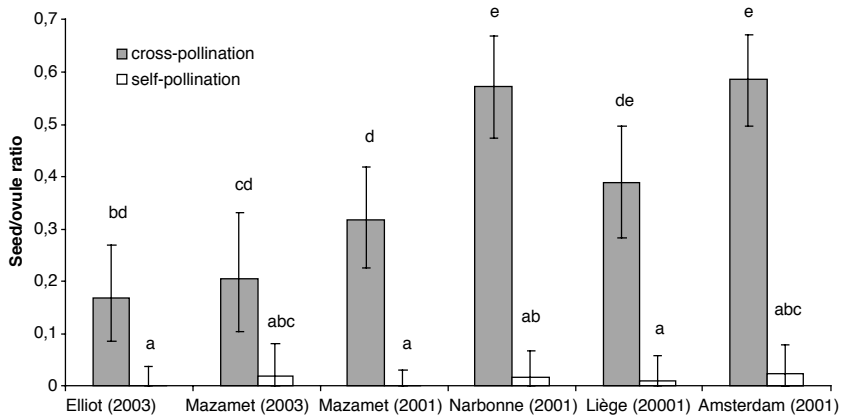

Fig. 2. Mean and confidence interval of the seed/ovule ratio of self- and cross-pollinations for each population. Data with the same letter do not show a significant difference according to a Tukey test for multiple comparisons. Due to mortality or male-sterility observed in some plants, final number of plants used in crosses are respectively: 8, 7, 10, 10, 8, 10.

compatible and incompatible crosses and no significant correlation between the self and cross-compatibility level of individuals ($p > 0.05$ for all tests).

Discussion

Reproductive system of *Senecio inaequidens*

This work first confirmed that *Senecio inaequidens* is a self-incompatible species (Lopez-Garcia and Maillet 2005): few selfing trials lead to a seed/ovule ratio higher than 0.1. Besides data obtained with two enzyme loci showed no significant departure from Hardy-Weinberg proportions indicative of no inbreeding in all European and South African populations studied (Lafuma 2003). The occurrence of reciprocal differences in cross-compatibility between some pairs of parent plants is a typical feature of sporophytic self-incompatibility (Hiscock and Tabah 2003). Results of reciprocal crosses thus gave some evidence that *S. inaequidens* possesses a homomorphic sporophytic self-incompatibility system, as do species of the Compositae studied so far and *Senecio squalidus,* a species of the same genus (Hiscock and Tabah 2003). We can not rule out the possibility that under some conditions the effectiveness of this system will be weakened. Nevertheless, preliminary experiments suggested that an increase in temperature (as for homomorphic self-incompatible system in *Brassica*, Hodgkin et al. 1988) or floral age (as for *Campanula rapunculoides*, Vogler et al. 1998) do not influence the seed set of self-pollinations in our system (Lafuma 2003).

Table 2. Number of outcrosses for the two seed/ovule ratio classes: a cross giving a seed/ovule ratio <0.1 is being considered as incompatible (see text). Comparisons (a) between a native and an introduced population (between plants intra-population crosses), (b) between old and recent populations of the same transect of colonization (between plants intra-population crosses) and (c) between intra-population crosses and inter-populations crosses.

Populations involved in the crosses	Year of the experiment	Seed set		$\chi 2$	p-value
		<0.1	≥ 0.1		
(a)					
Mazamet(♀) × Mazamet(♂)	2003	7	16		
Elliot(♀) × Elliot(♂)	2003	19	12	5.025	**0.025**
(b)					
Mazamet(♀) × Mazamet(♂)	2001	15	27		
Narbonne(♀) × Narbonne(♂)	2001	9	38	3.090	0.079
Liege(♀) × Liege(♂)	2001	13	24		
Amsterdam(♀) × Amsterdam(♂)	2001	10	50	4.315	**0.038**
(c)					
Mazamet(♀) × Mazamet(♂)	2001	15	27		
Mazamet(♀) × Liege(♂)	2001	4	24	3.901	**0.048**
Liege(♀) × Liege(♂)	2001	13	24		
Mazamet(♀) × Liege(♂)	2001	4	24	3.841	0.058

The level of self-compatibility did not differ between the African population and the older European population of France, nor between the older and the more recent populations in each invasion transect. This means that the self-incompatibility system was not broken when the plant established in Europe, nor during the colonization of France and Belgium/Netherlands. Several studies of self-incompatible species in Asteraceae found a majority of individuals strongly incompatible and some with a higher level of compatibility (reviewed by Nielsen et al. 2003) and that is what we also observed: four individuals showed partial self-fertility, one in each European population. This shows that variability for self-compatibility exists but that it has nevertheless not been selected. Why has compatibility not evolved and how has *S. inaequidens* become such a successful colonizing species all over Europe when its reproductive system is expected to prevent uniparental reproduction and to limit available mates?

Factors maintaining self-incompatibility systems in colonizing species

Despite "the need [. . .] for further field and experimental studies" claimed by Baker (1967), there is little experimental evidence that reproductive assurance plays a role in the evolution of reproductive system in colonizing species. Amsellem et al. (2001) have demonstrated a switch in the reproductive biology of *Rubus alceifolius* between its native range and islands where it has been introduced, from sexual seeds production to apomixis. Barrett et al. (1989) showed the dissolution of a heteromorphic self-incompatibility system in geographically marginal populations of *Eichhornia paniculata*. However, to the best of our knowledge, no documentation on the breakdown of a homomorphic self-incompatibility system has been yet demonstrated during a colonizing process. Several factors may explain why self-fertility is not so crucial in many colonizing species. First, as Carlquist (cited in Baker 1967) has noticed "the establishment of at least two propagules is not a severe disadvantage". There are many circumstances in which the disadvantage is reduced. Since many weed introductions are not simply due to chance but are related to human activities, they probably consist in several propagules. Recent reviews actually show that genetic traces of bottleneck in outcrossing alien species are usually not found (Bossdorf et al. 2005, Novak and Mack 2005). Second, perenniality, massive seed production and/or seed dormancy may also contribute to the maintenance of a self-incompatibility system (Pannell and Barrett 1998) because there is less need for contemporaneity in the arrival of propagules. Third, a nonspecialized pollination mechanism also lowers the mating disadvantage of self-incompatible species. These classical characteristics may explain why self-incompatibility is maintained in invasive populations of *S. inaequidens.* First since several trade wool sites were contaminated in the early 20th century, we expect a massive import of seeds in Europe. Considering ecological factors, *S. inaequidens* is perennial, may produce many seeds with variable dormancy (Lopez-Garcia and Maillet 2005), has a wide flowering period and possesses a generalist pollinating system. Occasional pseudo-self-compatible individuals of *S. inaequidens* can help to ensure reproduction (Levin 1996), but the fact that the frequency of partially-self-compatible individuals stays low implies that selection continues to favour the maintenance of self-incompatibility.

Increase in mate availability during invasion

One interesting finding of our study is the higher seed/ovule ratio in cross-pollinations in recent populations compared to old populations of the same transect of colonization, and in the old French population compared to the South African population studied. These differences were not due to differences in seed/ovule ratio of compatible crosses, but to the proportion of compatible crosses (Table 2). The hypothesis that the difference between populations could be due to difference in sampling distance (if individuals were closer in some populations, they may be more genetically related and share the same incompatibility alleles) can be ruled out as we found no significant differences in mean distance between incompatible and compatible crosses. Environmental conditions and especially temperature can influence fertility and compatibility (de Nettancourt 1977, Levin 1996). However, our crosses within a year have been done simultaneously for all populations and under the same conditions. Therefore, environmental conditions can not account for the differences found.

A plausible explanation for the higher number of compatible crosses in the old French population of Mazamet compared to the South African population could be that the population of Mazamet was founded by a mixture of several South African populations. Wool-trade history is not precisely known, but there was a major wool export port in South Africa in the early 20s, Port-Elizabeth, where wools from different provenances were probably put together. A mixture of several populations could lead to more S-alleles and thus a higher percentage of compatible mates.

However, this explanation can not account for the increase of compatible crosses found within both transects (with only a marginally significant difference in the French one). Evolution of new S-alleles in such a short period of time is highly unlikely (Charlesworth

2000). An alternative hypothesis is provided by a property of sporophytic incompatibility system. In such systems, S-alleles can show dominant or codominant interactions. A hierarchical dominance between alleles increases the number of available mates relative to that produced by co-dominance, permitting compatible crosses between individual sharing recessively expressed S-alleles (Byers and Meagher 1992, Vekemans et al. 1998). Dominance thus reduces the disadvantage of limited mate availability that could follow colonization without losing the benefit of selfing avoidance. We have found between 15% (Amsterdam) and 65% (Elliot) incompatible crosses in the different populations of *S. inaequidens* studied, but we have no precise picture of the number of S-alleles and interactions among them. However our incomplete diallel design realized in each population gives some hints of dominance interactions between alleles (i.e. reciprocal differences in cross-compatibility). A change in average level of dominance relationship between alleles can occur if 1) the frequencies of the different types of alleles are differently affected by the repeated colonization process or 2) the dominance properties of the alleles themselves are modified by selection. The first possibility is supported by an infinite alleles model by Schierup et al. (2000) that showed that the distribution of S-alleles dominance can be changed in a structured population compared to a panmictic population. Such an effect could also be found in a colonisation process. The second possibility has been invoked by Brennan et al. (2006) to explain the results of their crosses within and between British populations of the colonizing *Senecio squalidus*.

Conclusion

Despite the existence of variability for self-compatibility among individuals in *S. inaequidens* the self-incompatibility system has remained strong in all European populations studied. The colonization process of *S. inaequidens* probably started with a sufficient number of propagules and S-alleles to allow this maintenance and this outbreeding system may in turn help in maintaining high levels of genetic variation. The possibility of an evolution of dominance relationships between S-alleles during the invasion process calls for further theoretical and empirical work. Furthermore, current geographical expansion of *S. inaequidens* will probably soon permit gene flow between populations originating from independent introductions previously separated (e.g. southern France and Belgium, Netherlands and Germany). The higher compatibility of inter-population crosses (involving populations of different introductions) compared to intra-population crosses will probably further promote invasiveness of *S. inaequidens*.

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