*The following resources related to this article are available online at www.sciencemag.org
(this information is current as of March 25, 2010):*

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/327/5973/1648

Supporting Online Material can be found at:
http://www.sciencemag.org/cgi/content/full/327/5973/1648/DC1

This article **cites 32 articles**, 2 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/327/5973/1648#otherarticles

This article appears in the following **subject collections**:
Botany
http://www.sciencemag.org/cgi/collection/botany
Evolution
http://www.sciencemag.org/cgi/collection/evolution

17. E. M. Ha *et al.*, *Nat. Immunol.* **10**, 949 (2009).
18. This work was supported by the Intramural Research Program of the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, NIH. We thank A. Laughinghouse, K. Lee, T. Lehman, and R. Gwadz for insectary support; J. Kabat for assistance with confocal imaging; J. Ribeiro and J. Valenzuela for their comments and insight; and B. Marshall for editorial assistance. The

A Self-Incompatibility System Explains High Male Frequencies in an Androdioecious Plant

Pierre Saumitou-Laprade,[1]|| Philippe Vernet,[1] Christine Vassiliadis,[1]* Yves Hoareau,[1]†
Guillaume de Magny,[1]‡ Bertrand Dommée,[2]§ Jacques Lepart[2]

Androdioecy is a sexual system in which males co-occur with hermaphrodites, which have both male and female function. Stable androdioecy is rare in nature, and theory suggests that it requires that males sire more than twice as many offspring as hermaphrodites. In several members of the olive family (Oleaceae), androdioecy occurs with higher frequencies of males than predicted by theory. In *Phillyrea angustifolia* L., we found that high male frequencies can be maintained in natural populations because hermaphrodites belong exclusively to one of two self-incompatibility groups, and thus, each can fertilize only half of all pollen recipients. In contrast, males can pollinate all hermaphrodites. Thus, in this species, the reproductive disadvantage that males face due to the loss of female function is offset by the fact that all males are fully compatible with all pollen recipients.

Most plant species are hermaphroditic, and individuals have both male and female function. However, populations of hermaphrodites containing individuals with only male or female function have evolved independently in numerous lineages. These populations may represent an intermediate stage between a fully hermaphroditic mating system and dioecy, a mating system where all individuals have only male or female function (*1*). The most frequently reported intermediate stage in plants is gynodioecy, where females invade a hermaphroditic population via a male-sterility mutation, and the remaining hermaphrodites then evolve to become progressively more male (*2*). Dioecy also may evolve via female sterility,

resulting in an androdioecious population (*1*). However, theory predicts that the evolution of gynodioecy or androdioecy from hermaphroditism should be difficult because, unless accompanied by a means to double their fitness, male- or female-sterile individuals will have half the reproductive capability of hermaphrodites, which can utilize both pollen and ovules for reproduction (*1*, *2*).

In plants, gynodioecy is relatively common (*3*); its success is explained by nucleocytoplasmic conflict (*4*). Male sterility is encoded by mitochondrial variants that are maternally transmitted (*5*) and, therefore, do not suffer from the reproductive disadvantage linked to the loss of male function; in some cases, they may even benefit from a reproductive advantage if male resources are reallocated to female function (*6*–*9*). Moreover, selfing associated with inbreeding depression can make it easier for gynodioecy to evolve. In contrast, high frequencies of males in androdioecious populations cannot be explained, as most female sterility appears to be Mendelian. According to theory, to be maintained in the population, males have to sire more than twice as many offspring as the hermaphrodites with which they co-occur (*10*). An even greater advantage is required in partially self-fertilizing populations, because fewer ovules in hermaphrodite recipients will be available for outcrossing (*1*, *10*–*13*). Thus, a functionally androdioecious species is expected to exhibit low male

frequencies in populations, self-incompatibility (SI) or at least a low selfing rate, and a large siring advantage for males.

Several species that have been described as morphologically androdioecious show a mating system more akin to a dioecious species in which hermaphrodites do not have an efficient male function (*10*). Only four animal genera and eight plant species have actually been confirmed as androdioecious (*14*, *15*). As in other Oleaceae species (*16*, *17*), the mating system of *Phillyrea angustifolia* is unclear (*18*) because this species has female-sterile individuals at frequencies that can reach or even exceed 50% in natural populations (*19*). In theory, such high frequencies require (*1*, *11*) an infinite male advantage and that hermaphrodites reproduce via their female function only. Accordingly, it has been suggested that the high frequency of males encountered in *P. angustifolia* populations may actually indicate functional dioecy (*14*).

Controlled crosses (*20*) in open-field conditions (*21*) have not provided support for this theory: They demonstrate that hermaphrodites effectively have male function and that males enjoy only a low, insignificant fitness advantage (*20*, *21*), which cannot explain their high proportions. We performed controlled crosses in natural populations with hermaphrodites sired by pollen either from males or from hermaphrodites (*22*) (table S1) and verified the crosses using paternity analysis with polymorphic microsatellite markers (*21*, *23*). These crosses revealed that female sterility is dominant and transmitted exclusively by males. Using additional controlled crosses, we determined incompatibility phenotypes with a stigma test, where we scored the presence or absence of pollen tubes in the stigma using fluorescent microscopy (*20*) (Fig. 1). Testing 107 hermaphrodites from five Mediterranean populations separated by 10 to 1000 km (fig. S1), we identified two groups of self-incompatible hermaphrodites (G1 and G2): In each group, plants were incompatible with each other but compatible with the members of the other group (Fig. 1 and Table 1) with no exceptions (table S2).

In contrast, all crosses performed between males and G1 and G2 hermaphrodite recipients were compatible (table S3) (*20*). Cross-compatibility assessed by the stigma test was subsequently confirmed by fruit production (table S3). Thus, males exhibit consistent and exclusive compatibility with all hermaphrodites tested so far, indicating that sex-determining

[1]Laboratoire de Génétique et Evolution des Populations Végétales, UMR CNRS 8016, Université des Sciences et Technologies de Lille–Lille 1, 59655 Villeneuve d'Ascq CEDEX, France. [2]Centre d'Ecologie Fonctionnelle et Evolutive (CEFE)–UMR 5175 du CNRS, 1919 route de Mende, 34293 Montpellier CEDEX, France.

*Present address: Laboratoire d'Ecologie, systématique et évolution (ESE), Département Génétique et Ecologie Evolutives, UMR 8079, Bâtiment 360 and 362, Université Paris–Sud, 91405 Orsay CEDEX, France.
†Present address: 22 rue des Orangers, 30000 Nîmes, France.
‡Present address: University of Maryland Institute for Advanced Computer Studies (UMIACS), 296 Biomolecular Sciences Building, Room 3104H, College Park, MD 20742, USA.
§Present address: 294 chemin des Baumes, 34980 Montferrier-sur-Lez, France.
||To whom correspondence should be addressed. E-mail: pierre.saumitou@univ-lille1.fr

and incompatibility loci are genetically linked. Hence, even when males and hermaphrodites produce the same amount of pollen, males can potentially sire twice as many ovules as hermaphrodites. This fitness advantage may be even higher if males are able to reallocate their resources into higher pollen production and/or flower production (24).

The fact that only two incompatibility groups were detected in hermaphrodites ruled out the possibility of gametophytic genetic control of self-incompatibility (GSI) in *P. angustifolia*. In GSI, the incompatibility (denoted S) gene is expressed in the haploid pollen grain after meiosis; this incompatibility system requires a minimum of three alleles and, consequently, a minimum of three incompatibility types to be functional (25). Alternatively, in sporophytic genetic control of self-incompatibility (SSI), the S gene is expressed before meiosis in the diploid sporophytic tissue, and incompatibility can arise with only two alleles, with a complete dominance of one allele over the other. Therefore, we hypothesize that *P. angustifolia* has an SSI governed by an S-locus with two alleles, S2 and S1 (with S2 dominant over S1), producing the two incompatibility groups G1 and G2. We predict that one group represents the recessive homozygote S1S1, and the other the S1S2 heterozygote.

Diallelic incompatibility in a homomorphic, wind-pollinated species has never before been observed in a homostylous plant genus (where styles—the pollen-conducting tube between the stigma and the ovule of a flower—are the same length in all individuals), although it has been shown to occur in the case of distyly (where styles vary in length, generally linked to incompatibility loci). In theory, diallelic incompatibility evolves either in heterostylous plants (26) or from a homostylous self-compatible plant (27), which suggests the existence of—at least transient—diallelic incompatibility in a homomorphic species.

Phylogenetic analysis suggests that the ancestral status of Oleaceae is diploid and distylous (28). Derived allopolyploid taxa, including the Oleinae subtribe to which *P. angustifolia* belongs, underwent hybridization and genome doubling and are mainly homostylous (29). Hence, during the breakdown of heterostyly, which may have occurred through allopolyploidization or in relation to a change in pollination system (30), the diallelic incompatibility system appears to have been conserved. The strict association between male phenotype and the full compatibility with G1 and G2 hermaphrodites requires genetic linkage between a gene determining sexual development (e.g., inhibiting stigma development) and either the self-incompatibility locus itself (31) or a modifier of self-incompatibility located elsewhere in the genome. Lending support to this theory, the self-incompatibility gene has a dual role in self-incompatibility and pistil development (32).

Whatever its origin, the occurrence of diallelic incompatibility in a wind-pollinated species has generated conditions that have driven evolution toward an androdioecious mating system, with the appearance of and maintenance of male individuals at higher-than-predicted frequencies within populations. Nevertheless, the maintenance of a diallelic incompatibility system in hermaphrodites, which is not associated with morphological differences, is surprising because these systems are susceptible to rapid invasion by new incompatibility alleles, which will experience a strong negative frequency-dependent advantage. This is true even after androdioecy
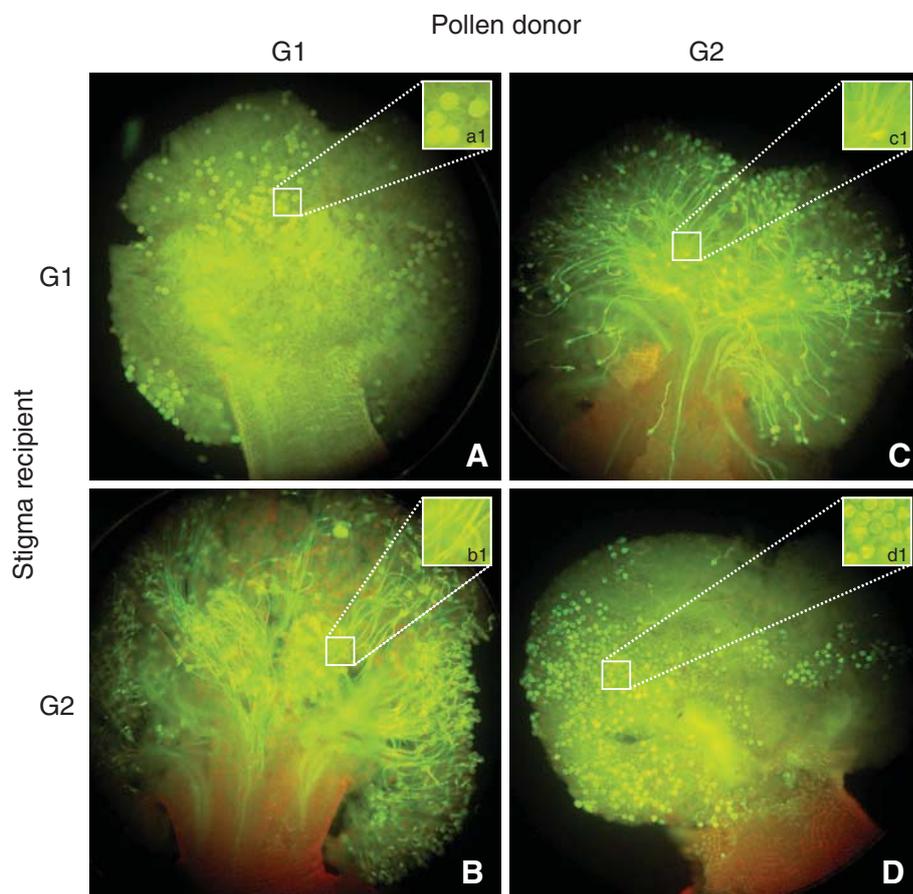

Fig. 1. Stigma tests. Stigmas of hermaphrodite recipients belonging to G1 are incompatible with pollen from G1 donors (**A**) but compatible with pollen from G2 donors (**C**). A strict symmetry is observed on stigmas of recipients belonging to G2: compatibility when pollinated by G1 (**B**), incompatibility when pollinated by G2 (**D**). (Insets) a1 and d1: zoom on nongerminated pollen grains; b1 and c1: zoom on pollen tubes.

Table 1. Two incompatibility groups detected among hermaphrodites sampled on a wide geographic scale: results of the stigma test. For numbered study sites, see map in fig. S1. Incompatibility groups in hermaphrodites are called G1 and G2; G1, hermaphrodites incompatible with each other and compatible with plants of the G2 group; G2, hermaphrodites incompatible with each other and compatible with plants of the G1 group. Of the 107 crosses performed, no cases of dual compatibility with both G1 and G2 were detected.

Study site	Number of hermaphrodite pollen recipients compatible with reference pollen donors		
	G1	G2	G1 and G2
1. Fabrègues	16	16	0/32
2. Moissac-Vallée-Française	10	9	0/19
3. Île Sainte-Lucie	9	10	0/19
4. Camargue–La Tour du Valat	16	2	0/18
5. Cadiz-Pinar de la Algaida	14	5	0/19
Total number of tested individuals	65	42	0/107

is established, because androdioecy itself does not favor the maintenance of only two incompatibility groups among hermaphrodites within populations. Investigations are now needed to determine the function and mechanism maintaining the low number of S alleles in *P. angustifolia* and any possible constraints that prevent the appearance of new incompatibility alleles.

Up to now, the high proportions of males in populations, sex determination, and relations among sexual phenotypes were inexplicable. This study at last provides an explanation by demonstrating the existence of unusual incompatibility relations among hermaphrodites. This incompatibility system may be more widespread, not only driving the diversification of the Oleaceae mating systems, but also other cases of distyly. This work supports previous hypotheses and models regarding the invasion of males in hermaphroditic populations (*10*, *25*, *33*). Until now, these theoretical ideas were largely contradicted by empirical examples of functional androdioecy that evolved from dioecious mating systems (*3*, *14*, *34*). Our results provide an alternative transition from hermaphroditism to androdioecy.

References and Notes
1. B. Charlesworth, D. Charlesworth, *Am. Nat.* **112**, 975 (1978).
2. D. Lewis, *Biol. Rev. Camb. Philos. Soc.* **17**, 46 (1942).
3. L. F. Delph, *Curr. Biol.* **19**, R249 (2009).
4. L. D. Hurst, A. Atlan, B. O. Bengtsson, *Q. Rev. Biol.* **71**, 317 (1996).
5. P. Saumitou-Laprade, J. Cuguen, P. Vernet, *Trends Ecol. Evol.* **9**, 431 (1994).
6. A. Atlan, P.-H. Gouyon, T. Fournial, D. Pomente, D. Couvet, *J. Evol. Biol.* **5**, 189 (1992).
7. D. Charlesworth, *Heredity* **46**, 27 (1981).
8. S. R. Keller, K. E. Schwaegerle, *J. Evol. Biol.* **19**, 1128 (2006).
9. P. Poot, *Am. J. Bot.* **84**, 1256 (1997).
10. D. Charlesworth, *Biol. J. Linn. Soc. Lond.* **22**, 333 (1984).
11. D. G. Lloyd, *Genetica* **45**, 325 (1975).
12. A. K. Sakai, S. G. Weller, M.-L. Chen, S.-Y. Chou, C. Tasanont, *Evolution* **51**, 724 (1997).
13. M. L. Van Etten *et al.*, *Int. J. Plant Sci.* **169**, 271 (2008).
14. J. R. Pannell, *Annu. Rev. Ecol. Syst.* **33**, 397 (2002).
15. S. C. Weeks, S. K. Reed, M. Cesari, F. Scanabissi, *Invertebr. Reprod. Dev.* **49**, 113 (2006).
16. B. Dommée, A. Geslot, J. D. Thompson, M. Reille, N. Denelle, *New Phytol.* **143**, 419 (1999).
17. K. Ishida, T. Hiura, *Int. J. Plant Sci.* **159**, 941 (1998).
18. J. R. Pannell, *Funct. Ecol.* **16**, 862 (2002).
19. J. Lepart, B. Dommée, *Bot. J. Linn. Soc.* **108**, 375 (1992).
20. C. Vassiliadis, J. Lepart, P. Saumitou-Laprade, P. Vernet, *Int. J. Plant Sci.* **161**, 393 (2000).
21. C. Vassiliadis, P. Saumitou-Laprade, J. Lepart, F. Viard, *Evolution* **56**, 1362 (2002).
22. Materials and methods are available as supporting material on *Science* Online.
23. P. Saumitou-Laprade, C. Vassiliadis, J. T. Epplen, C. Hardt, *Mol. Ecol.* **9**, 112 (2000).
24. J. R. Pannell, F. Ojeda, *Ecol. Lett.* **3**, 495 (2000).
25. C. Vassiliadis, M. Valero, P. Saumitou-Laprade, B. Godelle, *Heredity* **85**, 413 (2000).
26. D. G. Lloyd, C. J. Webb, in *Evolution and Function of Heterostyly*, S. C. H. Barrett, Ed. (Springer, Berlin, 1992), pp. 151–178.
27. D. Charlesworth, B. Charlesworth, *Am. Nat.* **114**, 467 (1979).
28. H. Taylor, *Brittonia* **5**, 337 (1945).
29. E. Wallander, V. A. Albert, *Am. J. Bot.* **87**, 1827 (2000).
30. J. H. Beach, K. S. Bawa, *Evolution* **34**, 1138 (1980).
31. V. Kurian, A. J. Richards, *Heredity* **78**, 383 (1997).
32. T. Tantikanjana, N. Rizvi, M. E. Nasrallah, J. B. Nasrallah, *Plant Cell* **21**, 2642 (2009).
33. J. R. Pannell, M. Verdú, *Evolution* **60**, 660 (2006).
34. L. F. Delph, D. E. Wolf, *New Phytol.* **166**, 119 (2005).
35. We thank A. Bourceaux and C. Collin for tending plants at Lille and Montpellier; C. Godé for help in genotyping; C. Raquin for advice in establishing pollen conservation methodology; F. Ojeda for providing samples from the Cadiz populations; A.-S. Blervaque for access to microscopy facilities and B. Buatois for access to biochemistry and microscopy facilities; M. Valero and J. Cuguen for financial support and encouragement; J. Pannell, P. O. Cheptou, I. Olivieri, V. Castric, X. Vekemans, M. Dufay, J.-F. Arnaud, S. Billiard, and V. Llaurens for scientific discussions and helpful comments on the manuscript; and A. Rodrigues and three anonymous referees for their helpful suggestions for improving the manuscript.

The Wnt/β-Catenin Pathway Is Required for the Development of Leukemia Stem Cells in AML

Yingzi Wang,[1] Andrei V. Krivtsov,[1] Amit U. Sinha,[1,2] Trista E. North,[3,4] Wolfram Goessling,[4,5,6] Zhaohui Feng,[1,2] Leonard I. Zon,[1,4,7] Scott A. Armstrong[1,2,4]*

Leukemia stem cells (LSCs) are capable of limitless self-renewal and are responsible for the maintenance of leukemia. Because selective eradication of LSCs could offer substantial therapeutic benefit, there is interest in identifying the signaling pathways that control their development. We studied LSCs in mouse models of acute myelogenous leukemia (AML) induced either by coexpression of the *Hoxa9* and *Meis1a* oncogenes or by the fusion oncoprotein MLL-AF9. We show that the Wnt/β-catenin signaling pathway is required for self-renewal of LSCs that are derived from either hematopoietic stem cells (HSC) or more differentiated granulocyte-macrophage progenitors (GMP). Because the Wnt/β-catenin pathway is normally active in HSCs but not in GMP, these results suggest that reactivation of β-catenin signaling is required for the transformation of progenitor cells by certain oncogenes. β-catenin is not absolutely required for self-renewal of adult HSCs; thus, targeting the Wnt/β-catenin pathway may represent a new therapeutic opportunity in AML.

Acute myelogenous leukemia (AML) is the most common acute leukemia in adults, and most patients are not cured with current therapies. Only a small subset of AML cells are capable of extensive proliferation and self-renewal (*1*). Such cells are referred to as leukemia stem cells (LSCs) because they share properties, including extensive self-renewal potential, with normal stem cells. LSCs are being studied as potential therapeutic targets (*2*, *3*), and the signaling pathways that control their development and survival are of particular interest. The Wnt/β-catenin pathway is active in certain human leukemias (*4–7*) and in normal hematopoietic stem cells (HSCs) (*8–10*). Although β-catenin is not required for self-renewal of adult HSC (*11*, *12*), it is unclear whether β-catenin is required for LSC development and maintenance in AML. We have studied well-characterized mouse models of AML to explore whether β-catenin is necessary for LSC development from either HSC or more differentiated committed myeloid progenitor cells.

Homeobox (*Hox*) genes have been implicated in the regulation of normal stem cell self-renewal (*13*, *14*), and enforced coexpression of *Hoxa9* and *Meis1a* in mouse bone marrow leads to rapid AML development (*15*). Previous studies demonstrated that leukemogenic fusion proteins, such as those involving the mixed lineage leukemia (MLL) protein (for example, MLL-AF9), can transform non–self-renewing granulocyte/macrophage progenitors (GMP) and activate

[1]Division of Hematology/Oncology, Children's Hospital, Harvard Medical School, Boston, MA 02115, USA. [2]Department of Pediatric Oncology, Harvard Medical School, Boston, MA 02115, USA. [3]Department of Pathology, Beth Israel Deaconess Hospital, Harvard Medical School, Boston, MA 02115, USA. [4]Harvard Stem Cell Institute, Harvard Medical School, Boston, MA 02115, USA. [5]Medical Oncology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA. [6]Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. [7]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA.

*To whom correspondence should be addressed. E-mail: scott.armstrong@childrens.harvard.edu