oceanic circulation (*25*). Source ages determined with the ^{129}I system in the Gulf Coast region (*12*) are also close to those of this study. If iodine ages determined in pore fluids associated with other gas hydrate deposits fall into the same time range, the case would be strengthened that the period around 55 Ma was responsible for large-scale deposition of organic matter and subsequent hydrocarbon generation in the oceans.

References and Notes

1. K. A. Kvenvolden, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3420 (1999).
2. E. D. Sloan, *Clathrate Hydrates of Natural Gases* (Dekker, New York, 1990).
3. W. Y. Xu and C. Ruppel, *J. Geophys. Res.* **104**, 5081 (1999).
4. J. T. Fabryka-Martin, H. Bentley, D. Elmore, P. L. Airey, *Geochim. Cosmochim. Acta* **49**, 337 (1985).
5. U. Fehn et al., *Geophys. Res. Lett.* **13**, 137 (1986).
6. D. R. Schink, P. H. Santschi, O. Corapcioglu, P. Sharma, U. Fehn, *Earth Planet. Sci. Lett.* **135**, 131 (1995).
7. U. Rao and U. Fehn, *Geochim. Cosmochim. Acta* **63**, 1927 (1999).
8. U. Fehn and G. Snyder, *Nucl. Instrum. Methods*, in press.
9. J. E. Moran, U. Fehn, R. T. D. Teng, *Chem. Geol.* **152**, 193 (1998).
10. P. K. Egeberg and G. R. Dickens, *Chem. Geol.* **153**, 53 (1999).
11. U. Fehn et al., *Geochim. Cosmochim. Acta* **56**, 2069 (1992).
12. J. E. Moran, U. Fehn, J. S. Hanor, *Geochim. Cosmochim. Acta* **59**, 5055 (1995).
13. W. C. Riese, S. G. Franks, U. Fehn, J. E. Moran, *Am. Assoc. Pet. Geol.* **1999**, A116 (1999).
14. G. T. Snyder and U. Fehn, *Nucl. Instrum. Methods*, in press.
15. G. T. Snyder, U. Fehn, F. Goff, *Eos* (West Pac. Geophys. Meet. Suppl.) **81**, 178 (2000).
16. D. R. Hutchison et al., *AAPG Mem.* **34**, 129 (1982).
17. R. Matsumoto and W. S. Borowski, *Proc. Ocean Drill. Program. Sci. Results* **164**, 59 (2000).
18. R. Hesse, S. K. Frape, P. K. Egeberg, R. Matsumoto, *Proc. Ocean Drill. Program Sci. Results* **164**, 129 (2000).
19. The pore water samples were squeezed from the sediments at shipboard following standard procedures and immediately sealed in glass bottles. Chloride was determined on shipboard by argentometric titration, and iodide and bromide concentrations were determined by the iodometric method and spectrophotometry (*10*).
20. Geochemical Earth Reference Model (2000), http://earthref.org/GERM/.
21. J. B. Martin, J. M. Gieskes, M. Torres, M. Kastner, *Geochim. Cosmochim. Acta* **57**, 4377 (1993).
22. Iodine was extracted from the samples and precipitated as AgI, with standard procedures (*11*). About 1 mg of iodine is needed to produce a reliable ^{129}I/I ratio with AMS. The samples yielded between 1 and 2 mg of AgI each, which was used for the isotope determination at the PrimeLab AMS system, Purdue University (*26*). As usual for isotopic dating systems, uncertainty in input ratio and the decay constant were not considered in the age calculations.
23. Determination of sediment and pore water age: Assume a constant rate of sediment deposition and steady state porosity profile $P(x) = (P_o - P_{inf}) \exp(-Lx) + P_{inf}$. Derive expression for sediment age: $F_s(x)$ (flux of sediment in kg m^{-2} year^{-1}) $= W(x)*R_s*S(x) = s$ (constant because of steady state) $=> W(x) = (s/R_s)S(x)^{-1}$ (m year^{-1}). The age of sediment particles $S_{age}(x)$ is

$$S_{age}(x) = \int_0^x W(x)^{-1} \, dx$$

Derive the expression for pore water age: F_w (flux of pore water in kg m^{-2} year^{-1}) $= V(x)R_wP(x) =>$ con-

stant (because of steady state). The constant is derived by the argument that at the depth of fully compacted sediment (x_{inf}), particles and pore water are buried at the same rate $=> V_{inf} = W_{inf}$. Hence, $F_w = W_{inf}R_wP_{inf} = V(x)R_wP(x) => V(x) = W_{inf}P_{inf}/P(x)$. The age of pore water $P_{age}(x)$ is

$$P_{age}(x) = \int_0^x V(x)^{-1} \, dx$$

The variables are as follows: P_o, porosity at sediment surface (0.72); P_{inf}, porosity at "fully compacted" sediment (0.43); $L = 0.002$; R_w, density of pore water (1000 kg m^{-3}); R_s, density of particles (2500 kg m^{-3}); s, rate of particle deposition at sediment surface (0.130 kg m^{-2} year^{-1}); $P(x)$, porosity profile; $S(x) = 1 - P(x)$, "sedimentosity" profile; $V(x)$, rate of pore water flow relative to sediment surface (m year^{-1}); and $W(x)$, rate of particle burial relative to sediment surface (m year^{-1}). Boundary conditions for sediment ages are derived from biostratigraphic data for Blake Ridge (*27*).

24. The addition of fissiogenic ^{129}I is described in the following equation:

$$N_{129} = (N_{238}Y_{sf}\lambda_{sf}\rho_r)(E/P)\{[1 - \exp(-\lambda_{129}t)]/\lambda_{129}\}$$

where N_{129} is the concentration of fissiogenic ^{129}I in the pore waters, N_{238} is the concentration of ^{238}U in the sediments, $Y_{sf}\lambda_{sf}$ is the product of the yield and the decay constant for spontaneous fission at mass 129, ρ_r is the density of sediments, E/P is the ratio of the escape efficiency to the effective porosity of the sediments, λ_{129} is the decay constant for ^{129}I, and t is the residence time of the pore water in the sediments. The following values were used for this calculation [see (*11*) for source references]: $\lambda_{sf} = 8.5 \times 10^{-17}$ year^{-1}; $Y_{sf} = 0.0003$; $\rho_r = 2.4$ kg/l; $E/P = 2$; and $\lambda_{129} = 4.4 \times 10^{-8}$ year^{-1}.
25. G. R. Dickens, M. M. Castillo, J. C. G. Walker, *Geology* **25**, 259 (1997).
26. D. Elmore et al., *Nucl. Instrum. Methods* **123**, 69 (1997).
27. H. Okada, *Proc. Ocean Drill. Program Sci. Results* **164**, 331 (2000).
28. We thank D. Elmore and P. Sharma for the ^{129}I/I determinations and J. Gieskes and R. Hesse for helpful comments. U.F. acknowledges the hospitality provided by the Beppu Geothermal Institute, Kyoto University, which allowed the timely completion of this report. The research was supported in part by NSF grant OCE-9907024 to U.F.

Polyploidy and the Evolution of Gender Dimorphism in Plants

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Gender dimorphism and polyploidy are important evolutionary transitions that have evolved repeatedly in many plant families. We show that gender dimorphism in North American *Lycium* (Solanaceae) has evolved in polyploid, self-compatible taxa whose closest relatives are cosexual, self-incompatible diploids. This has occurred independently in South African *Lycium*. We present additional evidence for this pathway to gender dimorphism from 12 genera involving at least 20 independent evolutionary events. We propose that polyploidy is a trigger of unrecognized importance for the evolution of gender dimorphism, which operates by disrupting self-incompatibility and leading to inbreeding depression. Subsequently, male sterile mutants invade and increase because they are unable to inbreed.

The evolution of sex and sexual systems is a central issue of evolutionary biology, and the deployment of sexual function into one or more morphs is a core concern (*1, 2*). Gender dimorphism (the presence of two sexual morphs in a population) occurs in only ~10% of angiosperm species but has evolved from cosexual ancestors in nearly half of the angiosperm families, making it an important evolutionary trend (*3*). Several pathways for the evolution of gender dimorphism have been advanced (*3–5*). The mechanistic explanations for the transition from cosexuality to gender dimorphism have concentrated on overcoming the inherent 50% fitness loss of single-sexed nuclear gene mutants arising in cosexual populations (*6, 7*). These mechanisms fall into two broad nonexclusive categories: elimination of inbreeding depression

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(IBD) by male sterile mutants (i.e., selection for outcrossing) and compensatory resource reallocation following the loss of one sexual function. Whereas both factors are thought to be important in the evolution of gender dimorphism, the outcrossing scenarios have more empirical support and are widely advanced as the principal mechanism favoring gender dimorphism (*8*). Because inbreeding avoidance is not important for self-incompatible taxa, the prominence accorded the inbreeding avoidance mechanism has led to a search for a correlation between the occurrence of separate sexes and an evolutionary background of self-compatibility (*9, 10*).

Polyploidy disrupts self-incompatibility in many species (*11, 12*). The tendency for polyploids to express self-compatibility [due to genic interactions in diploid pollen grains (*13*)] allows for the establishment of otherwise reproductively isolated polyploids (*11, 14*). Because polyploids buffer the effects of selfing more effectively than diploids (*11, 15, 16*), the resultant polyploid plants may be shielded initially

from the detrimental effects of IBD. However, theoretical (*17*) and empirical (*18, 19*) results indicate that equilibrium IBD under some conditions can be quite severe in polyploids. Specifically, for certain sets of dominance coefficients, IBD can vary non-monotonically with the selfing rate and even increase with increased selfing rates (*17*). In the face of strong IBD, male sterile mutants may invade because they produce no pollen and cannot self (Fig. 1). This potential connection between self-incompatibility, polyploidy, and gender dimorphism has not been widely appreciated [but, see (*20*)].

Polyploidy is associated with self-fertility and gender dimorphism in North American *Lycium* (wolfberry), as depicted in Fig. 2 (*21*). Gender dimorphism has evolved once among North American *Lycium* and occurs in three species that possess separate female and perfect-flowered (i.e., hermaphroditic) individuals. All other *Lycium* in North America (~18 species) are cosexual, with all individuals producing perfect flowers. Chromosome counts are available for 10 of the cosexual North American *Lycium*; all are diploid with $n = 12$ (*22*). The three dimorphic species, in contrast, are tetraploids or octoploids with $n = 24$ or 48 (*22*). Gametophytic self-incompatibility is well documented in Solanaceae (*12, 23, 24*). Allelic diversity at the self-incompatibility (*S*) gene in *Lycium andersonii*, a close relative of the dimorphic taxa (Fig. 2), has been estimated at >35 alleles, and coalescence analysis has shown that the *S*-allele lineages in this species are older than the genus as a whole, indicating that self-incompatibility is the basal condition for *Lycium* (*23*). Results of experimental pollinations reveal that, for three cosexual species, outcross pollen results in a 14- to 27-fold increase in seed production per flower, as compared to selfing (Fig. 3). Furthermore, pollen tube growth following outcrossing was more successful than that following selfing (Fig. 4), and evidence of the self-incompatibility reaction (i.e., thickened, irregular callose deposition and wandering pollen tube growth) was observed in self-pollinations. These results indicate that gametophytic self-incompatibility, not the theoretically expected self-compatibility, is the immediate ancestral breeding system of the dimorphic *Lycium*. Fruit and seed set levels in the hermaphroditic morphs of the dimorphic polyploids are too low to be used to test for self-compatibility. Yet, pollen tube growth following selfing and outcrossing of these plants is equivalent, suggesting that self-incompatibility has broken down (Fig. 4), as shown in other Solanaceae (*12, 25*). It is difficult to document the level of IBD in the hermaphroditic morphs of polyploid, dimorphic *Lycium* species because of low levels of fruit and seed set. However, flowers of *Lycium* do not possess other outcrossing mechanisms such as spatial or temporal segregation of gender function that would reduce selfing once self-incompatibility was disrupted. Also, selfing among different flowers on the same plant is likely to be high because of large floral displays (>500 flowers per plant are often open simultaneously).

Gender dimorphism in South African *Lycium* is evolutionarily independent of and morphologically distinct from that in North America (*26, 27*). As in North America, all six dimorphic species are polyploids ($n = 24$ or 36), whereas all of the cosexual species (19 species) with chromosome counts are diploid with $n = 12$ (*27*) and are undoubtedly self-incompatible, based on molecular studies of *S*-allele variation (*23*).

Polyploidy is widespread in plants (*14, 28*). If it leads to selfing and gender dimorphism even occasionally, it should be possible to find examples in the literature of polyploid, self-compatible, dimorphic species that are associ-

Fig. 1. Scenario for the evolution of gender dimorphism involving polyploidy. Polyploidy disrupts self-incompatibility in cosexual, diploid taxa. The resultant self-compatible polyploids are then subject to invasion by male sterile plants that are free from selfing and IBD. SC, self-compatible; SI, self-incompatible.
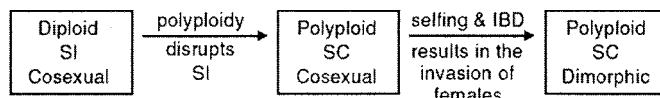
Fig. 2. Phylogenetic relationships, ploidy, and compatibility of North American *Lycium* (Solanaceae). Strict consensus of four trees that are most parsimonious, with a consistency index of 0.68 and a retention index of 0.70. Gender dimorphism has evolved once in North America (indicated by the arrow) and is associated with polyploidy and the loss of self-incompatibility. All other *Lycium* are cosexual in gender expression, having perfect flowers. Open bars represent the gender dimorphic, polyploid, self-compatible species, and hatched bars indicate cosexual, diploid species for which there is experimental evidence for self-incompatibility. All other species of *Lycium* are self-incompatible on the basis of transgeneric evolution of *S* alleles among *Lycium* and other genera in the Solanaceae (*23*). D, diploid; P, polyploid; SC, self-compatible; SI, self-incompatible. Asterisks indicate outgroup taxa, and bootstrap percentages are shown in association with nodes in the phylogeny.
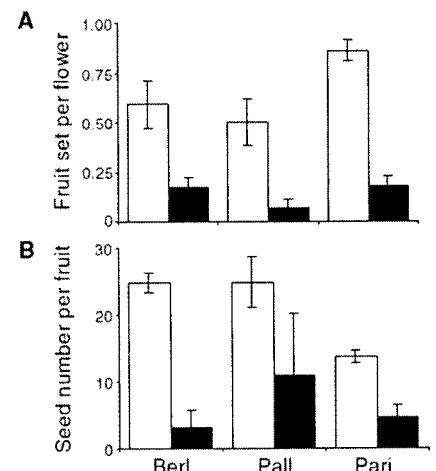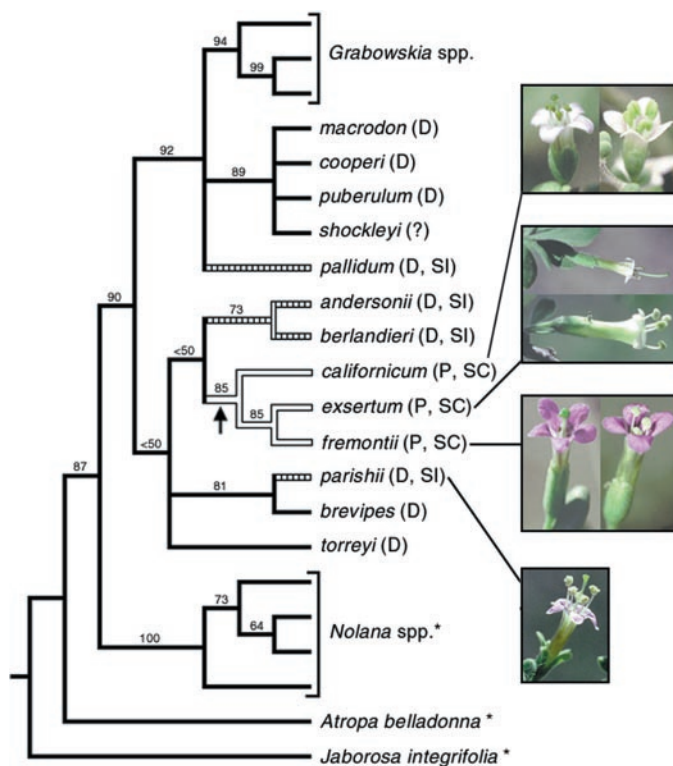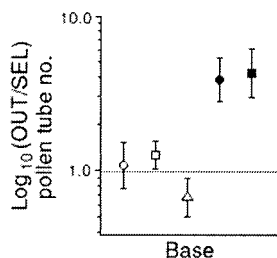

Fig. 3. Diploid, cosexual *Lycium* species are self-incompatible. Outcross pollination results in significantly higher (**A**) fruit set per flower and (**B**) seed number per fruit than does self-pollination in experimental crosses of three cosexual *Lycium* species. All comparisons between outcross and self were significant ($P < 0.005$), except for seed number in *L. pallidum*. Berl, *L. berlandieri*; Pall, *L. pallidum*; Pari, *L. parishii*. Error bars are ±1 SE. Details of methods are included as supplemental material (*29*).

ated with diploid, self-incompatible, cosexual ancestors. We have identified 12 genera in which polyploid, dimorphic taxa have evolved from diploid, cosexual ancestors at least 20 times (Table 1). In *Lycium, Chionographis, Rubus, Fragaria, Pachycereus,* and *Echinocereus,* the diploid taxa are cosexual and self-incompatible, whereas dimorphic taxa are polyploid and self-compatible. The relationship between polyploidy and dimorphism is further illustrated in *Potentilla, Mammillaria, Astilbe, Labordia, Thalictrum,* and *Bouteloua,* in which diploid taxa are cosexual and polyploid taxa are dimorphic. Data on compatibility systems are either incomplete or lacking for these groups but consistent with the proposed scenario where present.

A survey of 37 related pairs of taxa for which there is both compatibility information and variation in ploidy shows that a breakdown of incompatibility has occurred in 70% of polyploids associated with diploid self-incompatible plants (*29*). This suggests that the first step in the proposed scenario (Fig. 1) is widespread. Moreover, recent molecular work (*13*) and the experimental observation that induced polyploidy breaks down self-incompatibility (*11-13, 25*) argue for a causal role of polyploidy in the breakdown of incompatibility. Interestingly, of the subset showing the predicted pattern (i.e., diploids, self-incompatible and polyploids, self-compatible), 92% are in families known to have gametophytic self-incompatibility. Thus, the scenario outlined here may occur more often in species with gametophytically controlled self-incompatibility. Another test of the proposed scenario would be to determine compatibility for the genera listed in Table 1 for which information concerning compatibility is incomplete. In these cases, we predict that diploid taxa will be self-incompatible and polyploids will be self-compatible.

Fig. 4. Hermaphrodite morphs in polyploid, dimorphic species are self-compatible, whereas diploid, cosexual species are self-incompatible. Shown is the log ratio of outcross to self pollen tubes [i.e., the difference $\log_{10}$(outcross) − $\log_{10}$(self)] present at the base of the style for five *Lycium* species, including the hermaphrodite morphs of dimorphic *L. californicum* (open circle), *L. exsertum* (open square), and *L. fremontii* (triangle) and cosexual *L. pallidum* (solid circle) and *L. parishii* (solid square). Error bars are ±1 SE. Details of methods are included as supplemental material (*29*).

Though many polyploid self-compatible species may remain self-compatible cosexuals, selection for outbreeding will still exist in many such species and may frequently result in the evolution of new outcrossing mechanisms not present in the self-incompatible ancestors. The net results are likely to be various: purging and remaining predominant selfers, new spatial or temporal mechanisms that reduce selfing, or (as documented here) gender dimorphism. Determining the relative frequency of these outcomes following the breakdown of self-incompatibility would further reveal the scope of the proposed scenario.

Gender dimorphism has evolved by other avenues, and there are other patterns of gender dimorphism in relation to polyploidy and compatibility, yet these do not necessarily constitute counter evidence to the proposed scenario. For example, diploid *Empetrum nigrum* (Empetraceae) is dimorphic, whereas polyploid *E. hermaphroditum* is cosexual. Here, dimor-

phism evolved at the diploid level under an alternative evolutionary scenario, and the sex-determining mechanism appears to have broken down with polyploidy (*11*). Thus, polyploidy can break down dimorphism as well as trigger it. However, because gender dimorphism evolved on a diploid background before polyploidization, the importance of polyploidy as a trigger for gender dimorphism in self-incompatible, diploid, cosexual populations cannot be tested in such systems. Similarly, in *Isotoma fluviatilis* (Campanulaceae), diploid populations are either cosexual or dimorphic, whereas polyploid populations are exclusively cosexual (*30*). Apparently, both dioecious diploids and cosexual polyploids evolved from cosexual diploid populations. However, the diploids are self-compatible, so polyploidy is not a necessary trigger for gender dimorphism. No association is expected in such cases.

Baker (*9*) first suggested that dioecy and self-incompatibility are unlikely to exist togeth-

Table 1. Taxa in which gender dimorphism is associated with polyploidy. For each genus listed, the first line refers to the gender dimorphic taxa, and the second line refers to the presumed ancestral states of the dimorphic taxa. A fully referenced table is available as supplementary information (*29*). SC, self-compatible; SI, self-incompatible; P, polyploid; PF, cosexual plants with perfect flowers; D, diploid; ?, unknown; M, monoecious populations (i.e., cosexual plants with unisexual flowers). § indicates that dimorphic taxa cannot be self-pollinated to test for self-compatibility.

Taxon	Gender expression	Ploidy	SC/SI	Number of times evolved
Lycium (Solanaceae)	Gynodioecious (three species); dioecious (six species)	P	SC	≥2
	Cosexual (PF)*†	D (1 P)	D-SI (P-?)	
Chionographis japonica (Liliaceae)	Usually gynodioecious (three subspecies)	P	SC	≥1
	Usually cosexual (PF)‡	D	SI	
Rubus (Rosaceae)	Dioecious (five species)	P	SC	≥3
	Cosexual (PF)†	D or P	D-SI, P-SC	
Fragaria (Rosaceae)	Gynodioecious or dioecious (seven species)	P	SC or §	≥3
	Cosexual (PF, M)†	D	SI or SC	
Pachycereus (Cactaceae)	Trioecious (one species)	P	SC	1
	Cosexual (PF)†	D	SI	
Echinocereus (Cactaceae)	Functionally dioecious [one species with a few cosexual (PF) populations]	P	SC	1
	Cosexual (PF)†	D or P	D-SI	
Potentilla fruticosa (Rosaceae)	Dioecious populations	P	§	1
	Cosexual (PF) populations‡	D	SI	
Mammillaria (Cactaceae)	Gynodioecious (one species)	P	?	1
	Cosexual (PF)†	D or P	D-SI; P-SI	
Astilbe (Saxifragaceae)	Gynodioecious (one species)	P	SC	1
	Cosexual (PF)†	D	?	
Labordia (Geniostomaceae)	Dioecious (sixteen species)	P	?	1
	Cosexual (PF)*	D (*Geniostoma*)	?	
Thalictrum (Ranunculaceae)	Dioecious (nine species)	P	§	2
	Cosexual (PF)*†	D or P	?	
Bouteloua (Poaceae)	Dioecious (eight species)	P (1 D)	§	≥3
	Cosexual (PF, M)†	D or P	?	

*Characteristics of the closest known relatives of the dimorphic species, based on phylogenetic studies.   †Characteristics of cosexual species within the genus.   ‡Within-species example involving multiple populations or subspecies.

er in the same lineage because both are mechanisms to avoid inbreeding. The obvious corollary, that gender dimorphism is more likely to evolve in groups that are self-compatible, has often been discussed (*9, 10, 20*). Yet, if scenarios like that proposed here are common, gender dimorphism may frequently evolve in lineages with self-incompatibility without negating inbreeding avoidance as a selective mechanism. The pathway presented here reinforces the importance of inbreeding avoidance in the evolution of gender dimorphism and could explain why a negative association between gender dimorphism and self-incompatibility has been difficult to find (*10*). Although gender dimorphism has been widely studied, many aspects are not fully understood, and new scenarios, such as the one presented here, surely await discovery.

References and Notes

1. E. L. Charnov, J. Maynard Smith, J. J. Bull, *Nature* **263**, 125 (1976).
2. J. R. Kohn, *Nature* **335**, 431 (1988).
3. M. A. Geber, T. E. Dawson, L. F. Delph, Eds., *Gender and Sexual Dimorphism in Flowering Plants* (Springer-Verlag, Berlin, 1999).
4. K. S. Bawa, *Annu. Rev. Ecol. Syst.* **11**, 15 (1980).
5. J. D. Thomson and J. Brunet, *Trends Ecol. Evol.* **5**, 11 (1990).
6. D. G. Lloyd, *Genetica* **45**, 325 (1975).
7. B. Charlesworth and D. Charlesworth, *Am. Nat.* **112**, 975 (1978).
8. C. J. Webb, in (*3*), pp. 61–95.
9. H. G. Baker, *Cold Spring Harbor Symp. Quant. Biol.* **24**, 177 (1959).
10. D. Charlesworth, in *Evolution: Essays in Honour of John Maynard Smith*, P. J. Greenwood, P. H. Harvey, M. Slatkin, Eds. (Cambridge Univ. Press, Cambridge, 1985), pp. 237–268.
11. A. J. Richards, *Plant Breeding Systems* (Chapman & Hall, London, 1997).
12. D. de Nettancourt, *Incompatibility in Angiosperms* (Springer-Verlag, Berlin, 1977).
13. B. Chawla, R. Bernatzky, W. Liang, M. Marcotrigiano, *Theor. Appl. Genet.* **95**, 992 (1997).
14. J. Ramsey and D. W. Schemske, *Annu. Rev. Ecol. Syst.* **29**, 467 (1998).
15. B. C. Husband and D. W. Schemske, *Evolution* **51**, 737 (1997).
16. R. Lande and D. W. Schemske, *Evolution* **39**, 24 (1985).
17. J. Ronfort, *Genet. Res.* **74**, 31 (1999).
18. M. O. Johnston and D. J. Schoen, *Evolution* **48**, 1735 (1996).
19. D. R. Dewey, *Crop Sci.* **9**, 592 (1969).
20. H. G. Baker, *Am. Nat.* **124**, 149 (1984).
21. The internal transcribed spacers (ITS-1 and ITS-2) and the 5.8*S* cistron of nuclear ribosomal DNA were sequenced for 13 North American *Lycium* species, two *Grabowskia* species, and *Jaborosa integrifolia* following work by J. Wen and E. A. Zimmer [*Mol. Phylogenet. Evol.* **6**, 167 (1996)]. Sequences for *Grabowskia glauca* and four *Nolana* species were obtained from GenBank (accession numbers AB019954, AB019294, AB019311, AB019971, AB019314, AB019974, AB019966, AB019306, AB019289, and AB019949), and a sequence for *Atropa belladonna* was supplied by R. G. Olmstead. Sequences were aligned manually and combined with 29 morphological characters, producing a matrix of 733 characters, of which 17% were potentially phylogenetically informative. Phylogenies were inferred from 1000 random-addition sequence replicates with tree bisection reconnection (TBR) branch swapping using heuristic parsimony in PAUP* [D. L. Swofford, *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4.0b3a (Sinauer Associates, Sunderland, MA, 2000)]. Five hundred bootstrap searches, each with 50 random-addition sequence replicates and TBR branch swapping, were performed to assess the internal consistency of the data set. DNA sequences of this study are under GenBank accession numbers AF238981 through AF238995.
22. F. Chiang-Cabrera, dissertation, University of Texas, Austin (1981).
23. A. D. Richman and J. R. Kohn, *Plant Mol. Biol.* **42**, 169 (2000).
24. A. D. Richman, M. K. Uyenoyama, J. R. Kohn, *Science* **273**, 1212 (1996).
25. A. B. Stout and C. Chandler, *Science* **94**, 118 (1941).
26. J. S. Miller, in preparation.
27. A. M. Venter, H. J. T. Venter, R. L. Verhoeven, poster presented at the XVI International Botanical Congress, St. Louis, MO, 1 to 7 August 1999.
28. J. Masterson, *Science* **264**, 421 (1994).
29. Supplemental web material is available at www.sciencemag.org/feature/data/1053236.shl.
30. J. A. McComb, *Aust. J. Bot.* **16**, 525 (1968).
31. We thank L. McDade, J. Jaenike, R. Levin, and M. Nachman for comments on the manuscript and R. G. Olmstead for genomic DNAs (*Jaborosa integrifolia* and two species of *Grabowskia*) and a sequence for *Atropa belladonna*. This work was supported by grants from NSF (J.S.M. and D.L.V.), the University of Arizona Research Training Grant (J.S.M.), and Sigma Xi (J.S.M.).

Alteration of Stimulus-Specific Guard Cell Calcium Oscillations and Stomatal Closing in *Arabidopsis det3* Mutant

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Cytosolic calcium oscillations control signaling in animal cells, whereas in plants their importance remains largely unknown. In wild-type *Arabidopsis* guard cells abscisic acid, oxidative stress, cold, and external calcium elicited cytosolic calcium oscillations of differing amplitudes and frequencies and induced stomatal closure. In guard cells of the V-ATPase mutant *det3*, external calcium and oxidative stress elicited prolonged calcium increases, which did not oscillate, and stomatal closure was abolished. Conversely, cold and abscisic acid elicited calcium oscillations in *det3*, and stomatal closure occurred normally. Moreover, in *det3* guard cells, experimentally imposing external calcium-induced oscillations rescued stomatal closure. These data provide genetic evidence that stimulus-specific calcium oscillations are necessary for stomatal closure.

Cytosolic calcium ($[Ca^{2+}]_{cyt}$) oscillations are an integral component of cell signaling, and the frequency, amplitude, and spatial localization of oscillations control the efficiency and specificity of cellular responses in animals (*1–3*). In plant cells $[Ca^{2+}]_{cyt}$ oscillations are induced by multiple stimuli (*4–9*); however, it remains unknown whether oscillations are required to elicit physiological responses in plants. Here we show that the *Arabidopsis det3* mutant abolishes guard cell $[Ca^{2+}]_{cyt}$ oscillations and stomatal closure in response to oxidative stress and extracellular calcium ($[Ca^{2+}]_{ext}$), but not to abscisic acid (ABA) and cold. Restoring $[Ca^{2+}]_{ext}$-induced $[Ca^{2+}]_{cyt}$ oscillations in *det3* guard cells rescued stomatal closure, suggesting that $[Ca^{2+}]_{cyt}$ oscillations are essential for stomatal closure.

Stomatal closure follows increases in guard cell $[Ca^{2+}]_{cyt}$ (*10*), and endomembrane calcium transport contributes to the $[Ca^{2+}]_{cyt}$ signal (*7, 11–13*). Genetic impairment of endomembrane calcium transport could therefore provide a direct approach for dissecting $[Ca^{2+}]_{cyt}$ signals. The *de-etiolated 3 (det3)* *Arabidopsis* mutant has reduced endomembrane energization owing to a 60% reduction

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