curvature $\partial^2 M/\partial T^2 = \partial(C/T)/\partial H \cong (T_m \delta H)^{-1}[C(H + \delta H) - C(H)]$ would then show an oscillation with both positive and negative parts. This was not observed (Fig. 2B). The present measurements suggest a second-order transition, in agreement with recent results of torque magnetometry (*12*).

The amplitude of the step is $\Delta C = (6.6 \pm 15\%)$ mJ/(K gram atom). Ehrenfest's relation determines the corresponding change of slope of the equilibrium magnetization by $\Delta(\partial M/\partial T) = -(dH_m/dT)^{-1}\Delta C/T_m$. With $B_m = 4.2$ T, $T_m = 83.5$ K, and $T_c = 92.5$ K, we obtain $4\pi\Delta(\partial M/\partial T) = (0.20 \pm 15\%)$ G/K, to be compared with the experimental value $(0.2 \pm 25\%)$ G/K in the same field (*6*). The break in the slope is therefore confirmed to be an equilibrium property.

The amplitude of the step is nearly constant from 1 to 6 T. The number of vortices is proportional to B, and T_m decreases with B; therefore, the C jump per flux line increases when T_m approaches T_c. This may result from critical fluctuations (*3*), variations of the length of the correlated element in the flux line, or both. Below 1 T, the jump decreases and becomes sharper; above 6 T, it progressively transforms into a smooth crossover. Measurements at 14 T do not show any sharp structure on the extrapolated melting line. This sets an upper limit to the possible existence of a critical point terminating the melting line.

One of the signatures of a first-order transition is given by the phenomenon of superheating and supercooling. An observation of the heating and cooling rates in our experiment sets an upper limit of 0.2 K for the hysteresis. Preliminary measurements with an ac technique on an untwinned single crystal bring this limit down by an order of magnitude (*10*). No hysteresis was found in M measurements (*6*). These observations suggest a second-order transition.

The quantity $C(H,T) - C(0,T)$ was measured for Bi-2212 in fields that lie above the critical point [see figure 13a of (*13*)]. These data give an idea of the shape of the background near T_c in the absence of freezing. There is no positive overshoot such as that seen in Fig. 2A for Y-123.

The nature of the thermodynamic transition between the different vortex phases of high-temperature superconductors is still under discussion (*12*). Our measurements show a second-order step on the transformation line that had been determined independently by measurements of R and M. This second-order step constitutes the thermal signature of the melting of a vortex solid. This situation is analogous to the melting of a usual atomic lattice, with vortex modes playing the role of phonons (*14*). Our results establish that the transition line of interest and the break in the slope of M are equilibrium properties (*15*).

Note added in proof: Using differential thermal analysis, Schilling *et al.* (*16*) have observed a latent heat on the vortex lattice melting line of an untwinned Y-123 crystal. Using adiabatic calorimetry and a twinned, fully oxidized 18-mg single crystal grown in $BaZrO_3$, we observed a specific heat peak rising up to ≈1% above the C/T background in $B = 8$, 11, and 14 T on the vortex lattice melting line (*17*). The area under the peak corresponds to a melting entropy on the order of 0.5 k_B per vortex per layer up to the highest field. This result might confirm and extend Schilling's differential thermal analysis experiments.

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8. For example, the entropies
$$\int_T^{>T_c} dT[C(H) - C(0)]/T \text{ and } \int_0^H dH(\partial M/\partial T)$$
agree from $T = 120$ to 95 K in $\mu_0 H = 5.5$ T for the 116-K superconductor Tl-1223 [figure 27 of G. Triscone, A. Junod, R. E. Gladyshevskii, *Physica C* **264**, 233 (1996)], as expected in thermodynamic equilibrium. However, both estimations differ at lower temperatures, although M remains apparently reversible down to 65 K [G. Triscone and A. Junod, unpublished material]. The latter condition is not sufficient. This is also observed for the classical superconductor $Nb_{77}Zr_{23}$ [B. Revaz, unpublished material].
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29 April 1996; accepted 27 June 1996

Allelic Diversity and Gene Genealogy at the Self-Incompatibility Locus in the Solanaceae

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The self-incompatibility (*S*) locus of flowering plants offers an example of extreme polymorphism maintained by balancing selection. Estimates of recent and long-term effective population size (N_e) were determined for two solanaceous species by examination of *S*-allele diversity. Estimates of recent N_e in two solanaceous species differed by an order of magnitude, consistent with differences in the species' ecology. In one species, the evidence was consistent with historical population restriction despite a large recent N_e. In the other, no severe bottleneck was indicated over millions of years. Bottlenecks are integral to founder-event speciation, and loci that are subject to balancing selection can be used to evaluate the frequency of this mode of speciation.

Balancing selection can maintain large numbers of alleles within populations (*1*, *2*), and this polymorphism persists much longer than does selectively neutral genetic variation (*3*, *4*). At the *S* locus, the transmission rate of an allele is inversely proportional to its frequency, and populations commonly harbor as many as 30 to 50 alleles (*5*, *6*). Alleles at this locus can be extremely old, as reflected by their extreme sequence variability (*7*) and by phylogenetic analyses that have found that an allele from one species may be more closely related to an allele from another species or genus than to other alleles from the same species, a pattern called trans-specific evolution (*3*, *8*). Thus, historical events such as changes

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in population size leave an impression that persists much longer for variation subject to balancing selection than for neutral variation (*4*, *9*, *10*). At the major histocompatibility complex loci of vertebrates, extensive trans-specific variation is found in both the cichlid fishes in Lake Malawi (*11*) and in humans and other primates (*12*), which indicates that severe population constrictions, such as those invoked by models of founder-event speciation, cannot have been important during speciation of these taxa.

Two estimates of N_e can be derived from *S*-allele variability (*1*, *9*, *10*, *13*). The number of alleles present in a population reflects a short-term estimate because of strong selection for novel alleles in populations below equilibrium and rapid relaxation to an equilibrium of diversity after population restriction (*9*). In contrast, the number of trans-specific lineages inferred from allele genealogy evolves over millions of generations (*10*), because the loss of trans-specific lineages requires the extinction of all representatives of a lineage within a species. Nevertheless, in restricted populations the loss of alleles and of trans-specific lineages will be accelerated (*9*). After a bottleneck, newly generated alleles will lack the trans-specific evolutionary pattern, and the average sequence divergence among alleles within the species will be low

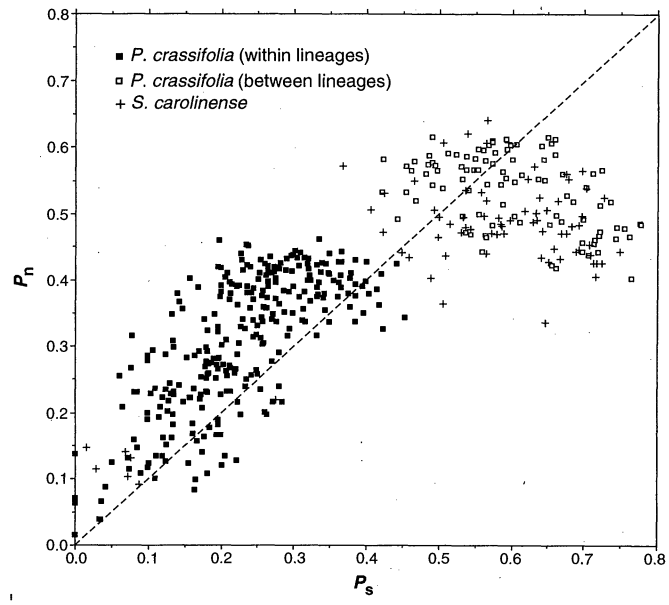

Fig. 2. Percent nonsynonymous substitutions per site P_n versus percent synonymous substitutions per site P_s for all pairwise comparisons within species, obtained with the program MEGA (*33*). Dotted line, 1:1 ratio of ordinate and abscissa; +, *S. carolinense*; ■, *P. crassifolia* within clade comparisons; □, *P. crassifolia* between clade comparisons.

Fig. 1. Aligned amino acid translations of partial *S*-allele cDNA sequences obtained by RT-PCR for *S. carolinense* (Sc) (*14*) and *P. crassifolia* (Pc) (*6*), 23 additional published *S*-allele cDNA sequences [*S. chacoense* (Schaco) (*34*, *35*), *S. tuberosum* (Stub S2) (*36*), *Lycopersicon peruvianum* (Lperu) (*37*– *39*), *Nicotiana alata* (Nalata) (*40*), *Petunia hybrida* (Phybrida) (*41*, *42*), and *Petunia inflata* (Pinflata) (*43*)], and two non-*S* ribonuclease sequences from *Lycopersicon esculentum* (LE) (*44*) and *Momordica charantia* (MC) (*45*). The initial alignment of amino acid sequences was obtained with the default settings of the multiple alignment program Clustal W (*46*) and was subsequently modified by eye. The first three positions include primer sequence for Sc and Pc sequences and were included for alignment purposes only. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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                1        10        20        30        40        50        60        70        80        90       100       110       120
MC      WPQ--GSGTSLTNCPQGS--PFDITK-ISHLQSQL-TLWPNVL----RANNQQ-FWSHEWITKHGTCSESTFNQA-AYFK---LVDMRNNYDIIGALRPHAAGPNGRTK--SRQAIKGFLKAKFG-KFPGLRC
LE      WFN-NNDGTYPSNCDPNS--PYDQSQ-ISDLISSMQQNWPTLAC--PSGSGST-FWSHEWEKHGTCAESVLTNQHAYFK--KALDLKNQIDLLSILQGADIHPDGES--YDLVNIRNAIKSAIG-YTPWIQC
PcS1    WPV-KKGEDNLMFCLPTP--NYTLFED-NKMLDDLDKHWMELKKRQKEGLEQQELWKRQYIKHGACCQNLYNQT-TYFS--LALSLKNRIDLLRNLRNHSIVP-GEN--YTFYEIAKAVKTVTG-ADSVFEC
PcS3    WPD-KKGVDKLTFCSAQP--NYTLFQD-KKMLDDLDKHWIQLKYSRENGLRKQEFWKRQYEKHGSCCLNRYNQT-AYFS--LALRLKDKIDLLSTLHNSGIDP-GEN--YTFQEIAKAIKTVTT-ADSLFKC
PcS27   WPD--KNGVQLNDCQPPP--NYTIIQ--DKMLIELDTHWTQLKIDKESGKKDQTIWKYRYIKHGSCCRELYNQS-MYFS--LALHLKHRVNLLNNLRTQQIFP-GGQ--YTLDEIVKAVK
PcS26   WPD--KNNSLLVDCLPPP--NYTNFH--NKMFDDLDKHWTQLKIFKKKAAIDQSTWSYQYI
PcS4    WPD--KNNSVLVECQPYR--GYTNFK--DNMLDELDIHWTQFKYDKSSGLKDQKTWRYQYRRHGTCCQELYNQD-MYFS--LALRLKRKVDLLRDLRQNGIAP-GGN--YTFADIIKAVKTVSI-SEPNIRC
PcS28   WPD--KNNSMLVDCQPYR--GYTNFK--DNMLNELDIHWTQFKEEKRSGLKDQKTWRYQYKRHGTCCQELYNQD-MYFS--LALRLKRKVDLLSNLRQNGIAP-GGN--YTFADIIKAVKTVSI-SEPNIRC
PcS5    WPD--KNGVQLNDCQPPP--NYTNIQ--NKMFDDLDTHWTQLKIDKETGKKDQPIWKYQYIKHGSCCRELYNQS-MYFS--LALHLKHRANLLNNLKTQQIFP-GGQ--YTLDEIVKAVKAVLK-SVPNIKC
PcS6    WPE-KRGQKIMVSCS-QV--NYTLFED-RKMLDDLDKHWIQLKVSKDKGLEQQESWKYQYEKHGACCRESYDQN-MYFS--LALRLYERFDLLSTLKNHSIVP-GGN--YTIQEIAKAIRNVTK-SDSDIKC
PcS7    WPD-KRGRKMMISCKPQV--NYTLFED-RNMLDVLDKHWIQLKVSKNESLVQQELWKRQYEKHGSCSQEVYNQN-MYFS--LALRLYERFDFLSTLQKHSIVP-GEN--YTIQEISKAIKTVTK-TDSDIRC
PcS8            KMMVSCSSQV--NYTLFED-RKMLDDLDKHWIQLKVKQEEALVKQDGWKYQYEKHGACSQKTYNQS-TYFS--LALRLYERFDLLSTLQKHSIVP-GEN--YTIQEIARAIKTVTNNTESDIKC
PcS9    WPV-KKGDNMLKFCPPDV--NYTLFKD-RKMLDVLDEHWIQLTVKKDEVPLNQELWKRQYEKHGACCQEDYNQN-MYFS--LALRLYERFDLLSTLQMHSIVP-GGN--YTIQEIAKAIKNVTK-SNSDIKC
PcS2    WPE-KRGDNMLKFCPPEV--NYTLFKD-RKMLDVLDEHWIQLTVKKDEVPLNQELWKRQYE
PcS10   WPE-KRGQKMMEFCPPQV--NYTLFED-RKMLDVLDEHWIQLKVKKDEIPVKQELWKRQYEEHGACCQEVYDQN-MYFN--LALRLYERFDILSTLQKHSIVP-GEN--YTIQEIAKAIKNVTK-AGSDIKC
PcS21   WPD--NISTTLNFCNG-V--TYKNVK--DEKLNKLYIRWPDLEVEEAVCKKDQKFWIKEYEKHGSCCEKTYNQE-QYFD--LAMGLKDKFDLTNSLGRYGIIP-GKS--YPVQTINNTVKAITQ-GFPRFLC
PcS11   WPD--KNNALLMDCRPPP--NYTNFPR-NKMFADLDKHWTQLKIKENNTETDQSTWSYQYIKHGACCQNLYDQN-MYFS--LALHLKDRVNLLTNLIKQKISP-GGQ--YTLDEIAKAVKAVLN-SVPNIKC
PcS12   WPD--KAGVTLMDCSPTP--NYTNFR--GKMLDDLDTHWTQLLLTKGTGLAEQRIWNYQFT
PcS14   WPE-KKGENMLILCRPEV--NYTLFED-RKMLDDLDKHWTQLKVKKDEALLKQDAWKYQYEKHGACCQKAYNQS-TYFS--LALRLYERFDILSTLEKHSIVP-GEN--YTIQEIAKAIKTVTT-SEFDIKC
PcS15   WPDNSEKAGLLVDCKLSS--NYTNLK--DKMLDDLDTHWTQLQIDKEQGRAAERIWKYQYV
PcS13   WPE-KKGENNLMFCKPTP--NYTLFED-KKMRDDLDKHWIQLKKRQKEGLEQQQFWKRQYIKHGACCQNLYNQT-TYFS--LALRLKDRIDLLRNLRNHSIVP-GEN--YTFYEIAKAVKTVTG-ADSVFEC
PcS16   WPDKSDRQGLLLGCKPPP--NYTKVK--DKMLDDLDKHWIQLLLKEKVGKTEQRIWKYQYMKHGSCCRELYNQD-MYFN--LALHLKDTVDLKRNLGKQNITP-GGN--YTFAEIIKAVKTVSK-SEPNIKC
PcS17         INNSVLMDCSPPL--NYTNFH--NKMFHELDKHWTQLKISKNNAEINQSTWSYQYL
PcS18   WPD--KHNESLMDCPPTP--NYTNFQ--NKMFADLDKHWTQFKILARNAQNDQSTWSYQYI
PcS19   WPE-KRGKKMMMSCKPEV--NYTLFED-RKMLDVLDKNWIQLKVSKNESLVQQELWKRQYE
PcS25   WPE-KRGQKMMMSCLPEV--NYTLFED-RKMLDDLDKHWIQLKVSKDKGLEQQEAWKYQYEKHGACSQESYNQN-MYFS--LALRLYERFDLLSTLQKHSIVP-GEN--YTIQEIAKAIKNV
PcS20       NFSAMLNFCG-PN--KYDKTILKDYKKNKLYILWPDLIVEEAKCKKDQKFWLDEYGKHGTCCEKSYSQE-QYFD--LAMVLKDKFDLLESFKRYGIIP-GTS--HTVQTINNTVKAITH-GFPNLSC
PcS22   WPD--NMSTTINFCG-PN--TYKKNIMDDDKKNNLYLRWPDLFVDEATCKKDQAFWKKEYG
PcS23   WPD--NISKPINFCH-RN--DYDSKLLKDYKKNNLYISWPDLFAEEADCKNGQKFWFDQYG
PcS24   WPE-EKGRNKLKFCKPLP--NYTLFED-KKMLDDLDKHWIQMKSSQDDGLQKQDLWKRQYL
LperuS3 WPD--KQRIMPINCPAKE--SYKSITD-SKKILKLLEQHWPDLTSNQGS----AEFWRYQYKKHGTCSVDLYNQE-QYFD--LAIELKEKFDLLKTLKNHGITP-SKTN--TVIDVEEAIKAVTK-EVPNLNC
LperuS7 WPD--HTDYIMYDCNPNK--EFKKIYD-KHLLNKLESRWPQLTSHEYAGLNDQTFWKYEYEKHGLCCEKVYDQS-QYFD--IAMKLKDSIDLLNILTTNRIVP-GFQYSYTGDQISSAIKRVTQ-KDPNPKC
LperuS6 WPD--NRSTILHDCDVPPEVDYVQIED-HKILNALDKRWPQLRYDYWYGIDKQYQWKNEPLKHGTCGINRYKQP-AYFD--LAMKIKDKFDLLGTLRKHGINP-GST--YELNDIERAIKTVSI-EVPSLKC
Lperul2a WPD--SEAGELNFCNPRA--SYTIVR--HGTFEKRNKHWPDLMRSKDNSMDNQEFWKHEYIKHGSCCTDLFNET-QYFD--LALVLKDRFDLLTTFRIHGIVP-RSS--HTVDKIKKTIRSVTG-VLPNLSC
Lperul3 WPD--HTSFVMYDCDPLK--KYKTIDD-TNILTELDARWPQLTSTKIIGLQFQRFWEYEYRKHGTCCADVFNQS-MYFD--ISMKLTDSIDLLKILRTKGIKP-GYT--YTGDQISRAIKSVTQ-NNPNPKC
StubS2  WPDKKPMRGQLQFCTSD---DYIKFTP-GSVLDALDHHWIQLKFERGIRDQPLWKDQYKKHGTCCLPRYNQL-QYFL--LAMRLKEKFDLLTTLRTHGITP-GTK--HTFKKIQDAIKTVTQ-EVPDLKC
SchacoS11 WPD-KEGPQLLKYCKPKL--TYNYFSD--KMLNDLDKHWIQLKIDQASARKDQPAWKYQYIKHGSCCQKIYNQN-TYFS--LALRLKDRFDLLRTLQIHRIVP-GS---STFEEIFDAVKTVTQ--MPDIKC
SchacoS2 WPD--NKKYLLNNCRSY---AYNALTN-VREQSKLDDRWPDLTSNKSMTMKEQKFWEYEYNKHGTCCEKLYNQA-QYFN--LTMNLKDKFDLLRILRNHGIVP-GSLA-LLSNSGRPLRQLTNK-VFPSLKC
SchacoS3 WPD--NTSTRLNFCKIV---KYNKIED-EHKIDALEYGWPNLTTTEAVSKEDQVFWGKQYTKHGSCCTDLYDKD-AYFD--LAMNLKDRFDLLKILAMHGITP-GTSH-HTSSNIQNAVKSVTQ-GVPHVTC
NalataS1 WPD--NVSTELNYCDRQK--KFKLFED-DKKQNDLDDRWPDLTLDRDDCKNGQGFWSYEYKKHGTCCLPSYNQE-QYFD--LAMALKDKFDLLKSFRNHGIIP-TKS--YTVQKYNNTVKAITK-GFPNLTC
NalataS2 WPD--NHTTMLNYCDRSK--PVNMFTD-GKKKNDLDERWPDLTKTKFDSLDKQAFWKDEYVKHGTCCSDKFDRE-QYFD--LAMTLRDKFDLLSSLRNHGISR-GFS--YTVQNLNNTIKAITG-GFPNLTC
NalataS3 WPD--NVSTMLNYCSGED--EYEKLDD-DKKKKDLDDRWPDLTIARADCIEHQVFWKHEYNKHGTCCSKSYNLT-QYFD--LAMALKDKFDLLTSLRKHGIIP-GNS--YTVQKINSTIKAITQ-GYPNLSC
NalataS6 WPD--NVSTTLNFCGKED--DYNIIMD-GPEKNGLYVRWPDLIREKADCMKTQNFWRREYIKHGTCCSEIYNQV-QYFR--LAMALKDKFDLLTSLKNHGIIR-GYK--YTVQKINNTIKVTKG--YPNLSC
NalataSZ WPD--KVRGRLQFCTSE---KYVNFAQDSPILDDLDHHWMELKYHRDFGLENQFLWRGQYQKHGTCCIPRYNQM-QYFL--LAMRLKDKFDLLATLRTHGITP-GTK--HTFNETRDAIKTVTNQVDPDLKC
NalataSa WPD--EQHGMLNDCGE----TFTKLE--PREKKELTIRWPDLKRSRSDAQDVESFWEYEYNKHGTCCTELYDQA-AYFD--LAKNLKDKFDLLRNLKNEGIIP-GST--YTVDECEKQSEAVTQ-AYPNLNC
NalataSF11 WPD--NVKTRLHNCKPKP--TYSYFT--GKMLNDLDKHWMQLKFEQDYGRTEQPSWKYQYIKHGSCCQKRYNQN-TYFG--LALRLKDKFDLLRTLQTHRIIP-GSS--YTFQDIFDAIKTVSQ-ENPDIKC
PinflataS1 WPE--ITGFRLEFCTGDP--KYETFKD-NNIVDYLERHWVQMKFDENYAKYHQPLWSYEYRKHGMCCSKIYNQK-AYFL--LATRLKEKFDLLTTLRTHGITP-GTK--HTFGDIQKAIKTVTNQVDPDLKC
PinflataS2 WPK--NKHFRLEFCTGD---KYSRFKE-DNIINVLERHWIQMRFDEKYASTKQPLWEHEYNRHGICCKNLYDQE-AYFL--LAIRLKDKLDLLTTLRTHGITP-GTK--HTFGEIQKAIKTVTNNKDPDLKC
PinflataS3 WPE--KEHFRLEFCDGD---KFVSFSLKDRIVNDLERHWVQMKFDEKFAKIKQPLWTHEYNKHGICSSNLYDQR-AYFL--LAMRVKDLDLLTTLRTHGITP-GTK--HTFGEIQKAIKTVTNNKDPDLKC
PhybridaSx WPD--NEQRRLQFCTST---EYSLF-D-GDILDDLDRHWIQLKFDKETGMQDQPLWHEQFRKHGTCCENRYKQM-PYFL--LAMRLKNKFDLLTTLRTHGITP-GTK--HTFDEIQKAIKTVTNQVDPDLKC
PhybridaS1B WPD--SISVIMNNCDPT---KTFATITEIKQITELEKKRWPELTTAQFALTSQSFWRYQYEKHGTCCFPVYSQS-AYFD--FAIKLKDKFDLLSILRSQGVTP-GST--YTGERINSSIASVTR-VKPNLKC
PhybridaS3 WPE--KKRFRLEFCTGD---KYKRFLEEDNIINVLERHWIQMRFDETYANTKQPLWEHEYNRHGICCKNLYDQK-AYFL--LAMRLKDKLDLLTTLRTHGITP-GTK--HTFGEIQKAIKTVTSNNDPDLKC
ScS1    WPD--NNSIILHDCPVDKKDRYFTITD-HKKLIALDKRWPQLKLDYFSGINDQDLWRHEFQKHGSCGIKRYKQ--AYFD--LAMKLKDKFDLLKILRNNGINP-GST--YHLKNIESAIMTVSG-KIPSLKC
ScS13   WPD--NKSIILHDCPIDKKDGYVTIRD-HRILTELDKRWPQLRHDYFTGINAQPHWRHEFEKHGTCGINRYKQP-AYFD--LAMKLKDKFDLLTVLRNHGIKP-GST--YLLKDIESAIMTVSI-KKPSLKC
ScS10   WPD--NNSIILHDCLIDKKDGYSTITD-HKILIELDKRWPQLRFDYFTGINAQPHWRYEFTKHGTCGVKRYKQP-AYFD--LAMKLKDKFDLLTVLRNHGIKP-GST--YLLKDIESAIMTVSI-KKPSLKC
ScS11   WPD--NVSTILHDCPVEKKDGYFTIKN-HKILIELEKRWPQLRYDYFTGINAQPHWKYEFL
ScS4    WPD--NKSQMLNDCSS-KK-RYHNILE-PDKRKQLEDEWPDLTAMAGDTEKHQKFWGYEFNKHGTCSIDLYNQE-AYFD--LAIKLKNQFDILKTLRNHGIIP-GKVS-TTVKNVEDAIKAVSA-HVPNLNC
ScS12   WPD--NKSTMLNDCYS--EDKYETIMD-PIKIKELMYYWPDLTSMDGDTQKHQAFWAYEFN
ScS2    WPD--EQHAMLNDC-G-KK--FNDIMD-PRESKELDKRWPDLKNRESIATKTQSFWRYEYNKHGTCCSERYNQK-EYFD--LAMNLKDKFDLLQILKSQGITP-GDSY--PVDKIEQAIRAVTH-EYPNLNC
ScS8    WPD--IKGTVLNNCNSQA--RYTPVT--GNDFDKRNKNWPDLFRTEADARKNQGFWRYEFVKHGTCCSDLFNEE-KYFD--LAVGLKDRFDLLKIFRNKGIIP-GSN--YTVNKIEKTIRTVTG-VVANLSC
ScS7    WPD--KLRRHLQFCTSD---KYIKFDP-GSLMDALDHHWIQLKYETEIGFNVQPLWRDQYVKHGTCCLPRYNQT-QYFL--LAMRLKDKFDLLTTLTHGITP-GTK--HTFKKIYDAIKTVTQ-NNPDLKC
ScS9    WPD--KEKRRLQFCTTTA---FKLFN--GAIRDNLDRNWIQLKYYQKPGLRDQPLWHEQYKKHGTCCEPRYNQM-QYFL--LALSLKDKFDLLATLRNHGITP-GTK--HPFDKIHDAIKTATHGINPDLKC
ScS3    WPD--HTSFIMYDCNKTI--KFKKIED-AAMLNKLVLRWPQLTSTESDASNDQPFWKYQFEKHGTCCSDVYSQS-VYFE--IAVKLKDSIDLLKILLTKRIIR-GFR--YTGDQISGAIKSVTQ-NDPNPKC
ScS5    WPD--KKGTLLQDCQPTP--QYTDFKD--KMLNDMDKHWIQLKVEKSIALAKQPSWKYQYRKHGACCQKVYDQN-AYFS--LALHLKDRFDLLRTLQIHRISP-GSS--YTFKEIMDAIKTVTQ-NVPDIKC
ScS6    WPD--NVSRTLNFCSG--KDDYKKLQE-EKEKNDLDERWPDLKTDKIDCIGGQVFWKNEYNKHGTCCSETYNRE-QYFD--LAINLKDKFDLLASLKKHGIIP-GNK--YTVQKINSTIKTITR-GYPNLSC
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relative to taxa that have not experienced a bottleneck event.

We used the reverse transcription polymerase chain reaction (RT-PCR) (*14*) to assay allele number and sequence variation at the *S* locus in two North American species of Solanaceae: the horsenettle *Solanum carolinense*, a herbaceous perennial weed of the southeastern United States and northern Mexico, and the ground cherry *Physalis crassifolia*, a perennial subshrub of southwestern deserts. All individuals were heterozygous, as expected at the *S* locus, and results of single-donor crosses demonstrated that the genetic transmission of the tested alleles was consistent with gametophytic self-incompatibility for both species (*6*, *14*). The sequences obtained (Fig. 1) span the hypervariable regions that are thought to play a role in self-nonself recognition (*7*).

Different numbers of alleles were recovered from the two species. Thirteen alleles were recovered from 26 heterozygous diploid genotypes sampled from two *S. carolinense* populations separated by ~250 km (14 genotypes from one population, 12 from the other). The 99% (asymmetric) confidence interval on the number of alleles in *S. carolinense* ranged from 13 to 15 (*15*). In contrast, 28 alleles were recovered from a sample of 22 *P. crassifolia* genotypes from a single population located within the University of California Deep Canyon Reserve (Palm Desert, California), yielding a population estimate of 43 to 44 alleles. Using the approach of Wright (*16*), we estimated the population sizes required to maintain these extents of *S* diversity to be 500 to 1000 for *S. carolinense* (*14*) and 6000 to 10,000 for *P. crassifolia* (*6*). Differences in *S* diversity among species likely reflect differences in ecological characteristics that affect N_e. *Solanum carolinense* is a clonal rhizomatous weed that occurs in small and probably short-lived patches, which suggests a small N_e, whereas *P. crassifolia* is a nonrhizomatous species that occurs over large areas of undisturbed desert habitat.

These species also differ in the extent of divergence among alleles. *Physalis crassifolia* alleles are more closely related on average than are alleles found in *S. carolinense*, which indicates more recent divergence in *P. crassifolia*. The difference in average divergence was assessed by comparing the mean length of terminal branches on within-species phylogenies of *S* alleles. The mean length of terminal branches in *P. crassifolia* (0.048, SE 0.014) is significantly shorter ($P < 0.05$) than in *S. carolinense* (0.13, SE 0.03) (*17*).

The two species also differ with respect to the numbers of nonsynonymous (P_n) and synonymous (P_s) substitutions per site (Fig. 2). Although most comparisons among alleles from *P. crassifolia* show an excess of nonsynonymous substitutions (consistent with positive selection for different specificities), most comparisons among *S. carolinense* alleles fail to show such an excess. This difference is related to the extent of divergence among alleles; failure to detect evidence for positive selection in more distant comparisons probably results from the accumulation of many synonymous substitutions over time (*18*, *19*). A similar pattern has been reported for *S* alleles in the sporophytic self-incompatibility system of Brassicaceae (*20*).

The greater average age of alleles in *S.*

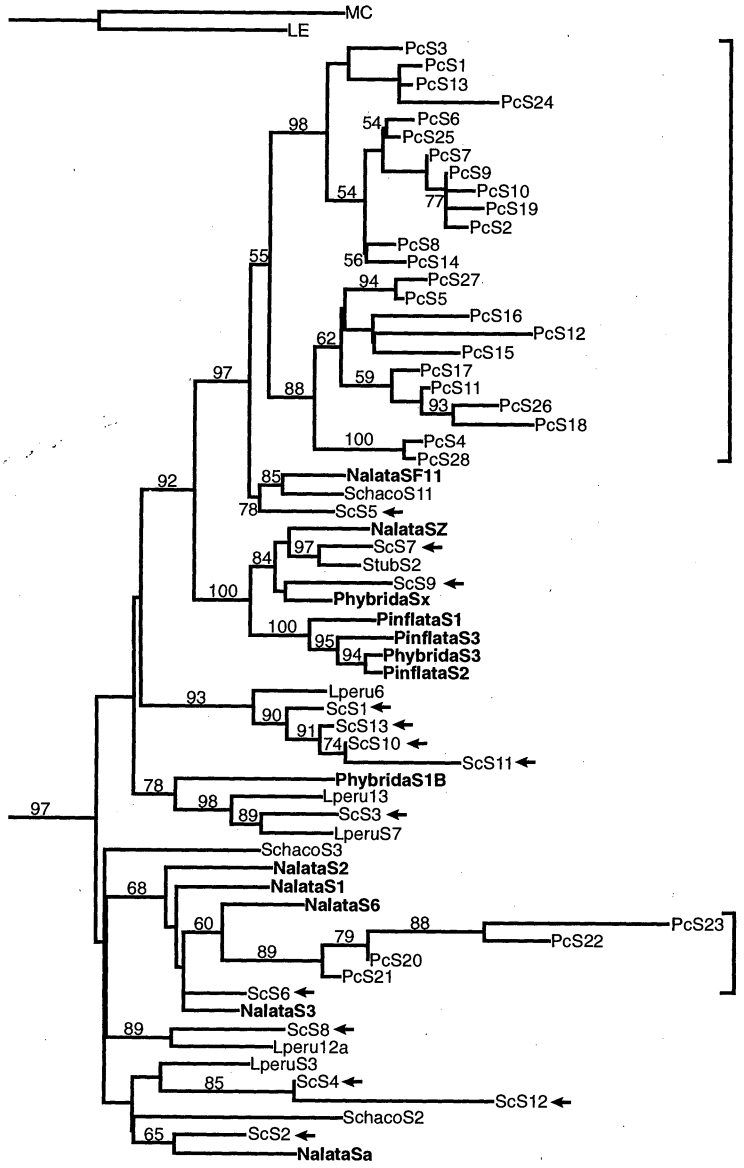

Fig. 3. Neighbor-joining tree based on amino acid distances between (partial) *S*-allele sequences from the Solanaceae. Distances were generated by means of the PAM Dayhoff similarity matrix implemented in the program Protdist in the phylogenetics analysis package Phylip 3.5 (*47*). Topology was determined with the use of the Phylip program Neighbor, and branch lengths were calculated by the least squares method implemented in Fitch (*47*). Numbers are bootstrap values exceeding 50%. Brackets indicate alleles from *P. crassifolia* (Pc) (*6*); arrows indicate alleles from *S. carolinense* (Sc) (*14*). Species names in bold denote *Nicotiana* and *Petunia* alleles. References for cDNAs included in the phylogenetic analysis are given in Fig. 1.

Table 1. Long-term N_e for *S. carolinense* and *P. crassifolia*, assuming origination rates (ν) that vary from 10^{-7} to 10^{-9} gene^{-1} generation^{-1} and different numbers of trans-specific lineages (k).

ν	N_e			
	S. carolinense (n = 13)		*P. crassifolia* (n = 28)	
	$k = 9$	$k = 8$	$k = 3$	$k = 2$
10^{-7}	6.5×10^5	3.9×10^5	1.5×10^4	0.6×10^4
10^{-8}	2.2×10^4	1.2×10^4	2.4×10^2	0.9×10^2
10^{-9}	3.0×10^2	1.3×10^2	0.7×10^2	0.6×10^2

carolinense is also reflected in the genealogy of S-allele sequences (Fig. 3). In *S. carolinense* there is extensive trans-generic evolution, with 9 of 13 lineages separated from the next closest conspecific lineage by an allele from *Petunia* or *Nicotiana*. By contrast, the *S* alleles in *P. crassifolia* fall into only two clades, such that only two lineages out of a possible 28 show a trans-specific pattern. The extensive trans-generic evolution observed in *S. carolinense* (*14*) and other species of Solanaceae (*8*) indicates that these lineages have been inherited through multiple speciation events. Against this background, the limited number of old lineages in *P. crassifolia* appears exceptional. One possible explanation for the paucity of trans-specific lineages in *P. crassifolia* is that the genus *Physalis* branched off early relative to other solanaceous taxa included in the phylogenetic analysis. However, molecular systematics of these genera unambiguously group *Solanum* and *Physalis* as sister derived taxa relative to *Nicotiana* and *Petunia* (*21*).

Long-term N_e was estimated from the extent of trans-generic polymorphism within species (*11, 22, 23*). We identified the number of lineages within each species that predate the time since divergence of *Nicotiana* from the clade containing *Solanum* and *Physalis*, which has been estimated at 30 million years ago (*8*). In *S. carolinense*, 9 of 13 lineages exceeded this age (*24*). This result is robust to uncertainty in the phylogeny; bootstrap resampling identified eight or more trans-generic lineages in 93 of 100 replicates. In *P. crassifolia*, all 100 bootstrap replicates contained three or fewer trans-generic lineages. The assumption that all lineages not showing the trans-generic pattern are younger than the time since divergence of the clade containing *Physalis* and *Solanum* from *Nicotiana* is most critical in application to *P. crassifolia*. This assumption appears reasonable in view of the relatively limited divergence of alleles within each of the major allelic clades (Fig. 2). We assume a generation time of 2 years for these short-lived perennials. This assumption is critical only if there has been a significant difference in generation time between these taxa over long evolutionary periods. Such a difference seems unlikely given that neither genus is remarkable in terms of life form variation, with most members consisting of short-lived perennials, similar to the taxa considered here. Another necessary assumption is an origination rate of new *S* alleles, which has been estimated within broad bounds. Studies of the frequency of mutations affecting self-incompatibility in pollen indicate that this parameter is $<10^{-7}$ gene^{-1} generation^{-1} (*25*). An origination rate of 10^{-9} gene^{-1} generation^{-1} is at odds with the extensive *S* diversity observed in small, isolated populations (*1, 10, 13, 26*).

Estimates of long-term N_e for various origination rates (ν) and numbers of ancient lineages (k) for *S. carolinense* and *P. crassifolia* (*23*) are shown in Table 1. For all parameter combinations, estimates of long-term N_e for *P. crassifolia* are smaller than the corresponding estimates for *S. carolinense*, usually by an order of magnitude or more, whereas estimates of recent N_e from the number of extant alleles yielded the opposite pattern. Although this result appears to leave unresolved the question of whether *P. crassifolia* has undergone an increase in population size or whether *S. carolinense* has undergone a reduction (or both), we suggest that the origination rate of 10^{-7} gene^{-1} generation^{-1}, which we treat as an upper bound, is probably too high. This rate gives a very large estimate of long-term N_e (3.9×10^5 to 6.5×10^5) in *S. carolinense*; the number of alleles predicted to occur in a population of this size is 338 to 444, much larger than available species-wide estimates in plants expressing gametophytic self-incompatibility (*14, 27*). For origination rates of $<10^{-7}$ gene^{-1} generation^{-1}, long-term N_e estimates for *P. crassifolia* are more than an order of magnitude smaller than the recent N_e estimated from the number of alleles in the Deep Canyon population. For origination rates of $<10^{-9}$ gene^{-1} generation^{-1}, long-term N_e estimates are very small for both taxa because little lineage extinction is expected with a low input rate of new alleles. However, this origination rate is unable to support the extensive *S*-allele diversity observed in small, isolated populations (*1, 10, 13, 26*). In view of the data on allele number, which indicate a large recent N_e for *P. crassifolia*, we conclude that patterns of lineage persistence within species are most likely explained by a historic population restriction that resulted in the loss of most *S*-allele diversity in *P. crassifolia*, followed by rediversification after the bottleneck event.

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15. The degree of overlap between population samples in *S. carolinense* was assessed according to

$$P(y; x,N_1,N_2,n_1,n_2) = \sum_{j=\max(y,n_1+x-N_1)}^{\min(x,n_1,N_2+y-n_2)} \frac{\binom{x}{j}\binom{N_1-x}{n_1-j}\binom{j}{y}\binom{N_1-j}{n_2-y}}{\binom{N_1}{n_1}\binom{N_2}{n_2}} \quad (1)$$

(*28*), where N_1 and N_2 are the numbers of alleles in two populations, x is the number estimated to occur in both populations, n_1 and n_2 are corresponding numbers of alleles sampled without replacement, and y is the number of alleles identified in both samples. Using data from (*6*), we obtain $N_1 = 12$, $N_2 = 14$, $n_1 = 12$, $n_2 = 11$, and $y = 10$. The maximum likelihood (ML) estimate of x is 12, indicating complete overlap between the two samples. Consequently, samples were pooled, and the number of alleles in the joint population was estimated with Paxman's ML estimator

$$n = N\left[1 - \left(1 - \frac{2}{N}\right)^r\right] \quad (2)$$

(*29*), where n is the number of alleles sampled from r individuals, and N is the estimated total number of alleles. For $r = 26$ and $n = 13$, $N = 13$ to 14. This estimator assumes equal allele frequencies, as expected under gametophytic self-incompatibility, and this assumption was tested with Mantel's (*30*) χ^2 statistic:

$$\chi^2_{n-1} = \frac{(n-1)(\Sigma C_j^2 - 4r^2/n)}{2r - 4r/n} \quad (3)$$

where C_j is the number of times an allele occurs, n is the number of alleles found, and r is the number of plant genotypes sampled. No significant deviation from equal frequencies was detected in *S. carolinense* ($\chi^2_{12} = 10.36$, $P > 0.5$). A significant but marginal deviation was detected in the *P. crassifolia* sample. A less biased estimator when allele frequencies are unequal gave the same estimated number of alleles (*6*).
16. The population size required to maintain a specified number of alleles was estimated by

$$n_a = \int_{1/2N}^{1} \phi(x)dx \quad (4)$$

(*13*), where

$$\phi(x) = 4N\nu^{2Nax}(1-2x)^{2Nb-1}x^{-1} \quad (5)$$

($0 < x < 1/2$), $\phi(x)dx$ is the number of alleles whose frequencies are from x to $x + dx$, $a = 1/[(1-J)(1-2J)]$, $b = 1/[2(1-J)] + \nu$, N is the effective population size, ν is the origination rate, and J is the effective homozygosity obtained as a solution of

$$\nu\sqrt{8\pi N}\exp\left\{\frac{2NJ}{[(1-J)(1-2J)]}\right\} = (1-J)^{-1/2}(1-2J)^{-N[1/1-J)+2\nu]} \quad (6)$$

N was determined for origination rates varying from 10^{-7} to 10^{-9} gene^{-1} generation^{-1} by setting n_a equal to the estimated number of alleles in the population, determined from Eq. 2.
17. The average divergence of alleles within species was assessed by means of a generalized least squares (GLS) approach for estimating branch lengths (*31*). Pairwise sequence distances were estimated with the Kimura two-parameter model, and the topology was determined by neighbor joining. The GLS criterion was used to test for evidence of rate changes within the tree. In implementing the method, one or more closely related *P. crassifolia* sequences were omitted to reduce collinearity. Sequences used to test the single-rate model were *S. carolinense* sequences ScS1–13 and *P. crassifolia* sequences PcS1–6, PcS8, PcS20, and PcS22. The overall fit of the model of a single rate for both species' sequences was quite good [goodness-of-fit χ^2 (190 df) = 178, $P > 0.5$]. Accordingly, intraspecific phylogenies of allelic sequences were constructed, and the means and variances of the lengths of terminal branches were estimated. Again, one or more closely related sequences in *P. crassifolia* were omitted. Because *P. crassifolia* sequences are in general more

closely related, this procedure resulted in a conservative test. Sequences examined in *P. crassifolia* were sequences PcS1–9, PcS11–13, PcS16, and PcS20–22.
18. Tests for positive Darwinian selection (an excess of P_n relative to P_s) were performed using the approach of Nei and Jin (*32*) as implemented in MEGA (*33*). DNA sequences used in this analysis have been deposited in GenBank [accession numbers L40539 to L40551 (*15*) and L46653 to L46680 (*6*)]. Evidence for positive selection may be obscured by the accumulation of neutral mutations when more distantly related alleles are compared. Accordingly, P_n and P_s (and their SEs) were calculated separately for each major clade of *P. crassifolia* alleles (Fig. 3). To maximize the number of sequences analyzed, we used sequence data corresponding to residues 1 through 59 (Fig. 1). This region contains the two hypervariable regions (*7*), which may play a role in specificity determination. For the large clade of alleles from *P. crassifolia* (sequences 1 to 19 and 24 to 28), the mean values (with SEs) were P_n = 0.3082 (0.0227) and P_s = 0.2278 (0.0411); P_n/P_s = 1.47 ($P < 0.05$, one-tailed). For the small *P. crassifolia* clade (sequences 20 to 23), P_n = 0.2489 (0.0308) and P_s = 0.1855 (0.0569); P_n/P_s = 1.41 (not significant). For the four closely related alleles in *S. carolinense* (ScS1, ScS10, ScS11, and ScS13; see Fig. 3), P_n = 0.1214 (0.0296) and P_s = 0.0581 (0.0296); P_n/P_s = 2.09 [$P < 0.05$, one-tailed; see also (*14*)]. Comparisons among the remaining nine more distantly related *S. carolinense* alleles showed no excess of P_n [P_n = 0.4916 (0.0293), P_s = 0.5495 (0.0599); P_n/P_s = 0.89 (not significant)], similar to values found when comparisons were made across the two clades of alleles in *P. crassifolia* (P_n/P_s = 0.90).
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23. In calculating the probability $g_{nk}(t)$ that, given a sample of n extant alleles, they coalesced into k lineages t generations ago, it is convenient to replace t by t', defined as

$$t' = \frac{t}{2N_e f_s} \quad (7)$$

(*9*), where f_s is a scaling factor specific to frequency-dependent selection at the *S* locus, defined as

$$f_s = \frac{\sqrt{2}}{16N^2 \nu a (J - \nu/a)^2} \quad (8)$$

(*10*), where ν, J, and a are defined as in (*16*). The probability $g_{nk}(t')$ is then

$$g_{nk}(t') = \sum_{m=k}^{n} \frac{(2m-1)(-1)^{m-k} k_{(m-1)} n_{[m]}}{k!(m-k)! n_{(m)}} \exp\left[-\frac{m(m-1)t'}{2}\right] \quad (9)$$

for $2 \le k \le n$, where $n_{[m]} = n(n-1)(n-2)\ldots(n-m+1)$, $n_{(m)} = n(n+1)(n+2)\ldots(n+m-1)$, and m is a variable changing from k to n (*9*). For a given origination rate, we then calculate the probability $g_{nk}(t')$ to find the value of t' that maximizes the probability of observing n and k. The value of N_e associated with the ML estimate of t' is then obtained from Eq. 7 using $t = 15 \times 10^6$, assuming that *Nicotiana* diverged from *Physalis* and *Solanum* 30 million years ago and a generation time of 2 years (see text).
24. An *S* lineage was considered trans-generic if it inserted into the *S*-gene genealogy at a position ancestral to an allele found in *Nicotiana* [or *Petunia*, a more distantly related genus in the Solanaceae (*21*)]. The sensitivity of this estimate to uncertainty in the phylogeny was examined by use of the bootstrap. The data were resampled 100 times with replacement, and the number of trans-generic lineages was determined for each replicate.
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15 March 1996; accepted 31 May 1996

Forskolin Stimulation of Water and Cation Permeability in Aquaporin1 Water Channels

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Aquaporin1, a six–transmembrane domain protein, is a water channel present in many fluid-secreting and -absorbing cells. In *Xenopus* oocytes injected with aquaporin1 complementary RNA, the application of forskolin or cyclic 8-bromo- adenosine 3′,5′-monophosphate increased membrane permeability to water and triggered a cationic conductance. The cationic conductance was also induced by direct injection of protein kinase A (PKA) catalytic subunit, reduced by the kinase inhibitor H7, and blocked by $HgCl_2$, an inhibitor of aquaporin1. The cationic permeability of the aquaporin1 channel is activated by a cyclic adenosine monophosphate–dependent mechanism that may involve direct or indirect phosphorylation by PKA.

Lipid bilayers have an inherently low water permeability, an attribute that benefits life in aqueous and terrestrial environments. In specialized cells, water permeability is enhanced by the expression of aquaporins, integral membrane proteins that regulate osmotically driven transmembrane water fluxes (*1*). The primary sequences of aquaporins predict six transmembrane domains and internal NH_2- and COOH-terminal domains (*2*). This structural motif is similar to that of other channels and transporters (*3*).

In *Xenopus* oocytes, expression of complementary RNA (cRNA) encoding human aquaporin1 (CHIP28) confers an increased osmotic water permeability (*4*). Aquaporins 2 and 5 have consensus sites for the adenosine 3′,5′-monophosphate (cAMP)–dependent PKA (*5*). Aquaporin2, the vasopressin-regulated water channel, shows a cAMP-dependent increase in water permeability when expressed in *Xenopus* oocytes (*6*). In contrast, aquaporins 1 and 3 lack typical consensus sequences for phosphorylation by PKA and are thought to be constitutively active.

Voltage-clamp studies of *Xenopus* oocytes with aquaporin1 cRNA provide no evidence for ionic permeability (*4*, *7*). We also have found that the unstimulated aquaporin1 channel shows no evidence of net ionic flux. However, after treatment with forskolin, which increases production of cAMP by adenylyl cyclase, the rate of osmotically driven water uptake in aquaporin1-injected oocytes (*8*) was increased (Fig. 1). Swelling was quantitated by videomicroscopy after exposure of an oocyte to hypotonic saline (100-mOsm gradient). Oocytes incubated for 15 min in isotonic saline containing 10 μM forskolin showed the greatest subsequent rate of swelling in hypotonic saline. Ethanol (0.1%), used for dissolving forskolin, had no effect alone on swelling. Unstimulated oocytes expressing aquaporin1 showed an intermediate rate of swelling; their initial swelling rate was decreased 66% by $HgCl_2$ (100 μM), a blocker of water channels (*9*, *10*). Water-injected control oocytes showed a low swelling rate that was unaffected by forskolin. Calculated coef-

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