tectonic events[9,31,32] at any portion of the ridge would be expected to introduce additional complications. We speculate that the surface lavas at the axial high represent the waning stages of a voluminous magma pulse and may be younger, by 10^2–10^3 years, than lavas elsewhere along the axis. A similar interpretation has been made recently for the along-axis high at the MAR at 26° N, on the basis of micro-earthquake and refraction data[33].

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Why be female?

Joshua R. Kohn

Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

The evolution of dioecy (separate males and females) from hermaphroditism in higher plants has puzzled evolutionary biologists since Darwin[1]. An initial step towards the evolution of dioecy may often be the evolution and persistence of females within otherwise hermaphroditic populations[1–6]. This step is difficult because a nuclear gene for being female must provide a greater than twofold increase in female fitness for females and hermaphrodites to coexist stably[5–8]. It is unlikely that seed production would increase this much as a result of not producing pollen. Here I show that in buffalo gourd, seeds from females survive their first year in nature 2.8 times more frequently than seeds from hermaphrodites, apparently because seeds from hermaphrodites are mostly self-fertilized and selfing severely reduces seedling survival.

Buffalo gourd, *Cucurbita foetidissima*, a perennial plant native to the southwestern USA and northern Mexico, inhabits disturbed sites, chiefly roadsides. Plants annually produce from one to 30 stems, each up to 12 m long, and can propagate vegetatively by producing roots at leaf nodes. Hermaphrodite (monoecious) plants produce separate male and female flowers, female (gynoecious) plants produce female flowers only. Gender appears to be a mendelian trait, with females the heterogametic sex (refs 9 and 10; and J.R.K., unpublished results). Populations of buffalo gourd contain on average 32% females and 68% hermaphrodites, and females produce 1.5 times as many seeds as hermaphrodites (J.R.K., in review), an increase which is insufficient to explain their existence, especially at their current frequency[5–8]. Hermaphrodites are self-compatible, and electrophoretic analysis of seed allozymes indicates that most of their seeds result from self- (or intra-genet) fertilization (J.R.K. and B. B. Casper, manuscript in preparation).

Hand self- and cross-pollinations were performed on hermaphrodite plants in June of 1985. Only one hermaphrodite from any patch was used as a pollen recipient, and cross-pollinations were made using a male flower from another patch to ensure that the pollen was from a different genet. Fruits were collected a minimum of 45 days after pollination and fruits from naturally pollinated flowers of the same hermaphrodite plants and from female plants were also collected. Seeds were removed from the fruits, dried at room temperature and counted.

Among hermaphrodites, cross-pollinations produced more seeds than either self- or natural pollinations, which did not differ (means: cross, 277; self, 184; natural, 168; $n = 15$ plants, $F = 11.6$, $P < 0.001$). Thus a potential cost of self-fertilization is reduced seed set; however, naturally pollinated fruits of females contain 14% fewer seeds than do those of hermaphrodites (J.R.K., in review), even though fruits of females must result from outcrossing. Females may be pollen-limited, but flowers of females are slightly smaller than female flowers of hermaphrodites (J.R.K., in review), so females may also have fewer ovules per flower.

Seed mass did not differ between hand self- and cross-pollinations, but both resulted in lighter seeds than natural pollinations (means, in g per seed: self-, 0.0434; cross-, 0.415; natural, 0.0473; $F = 5.6$, $P < 0.01$). This may be due to the harvesting of fruits before seeds were completely mature. Under experimental cultivation, buffalo gourd seeds reach full weight and germinability

* Present address: Botany Department, University of Toronto, Ontario M5S 1A1, Canada.

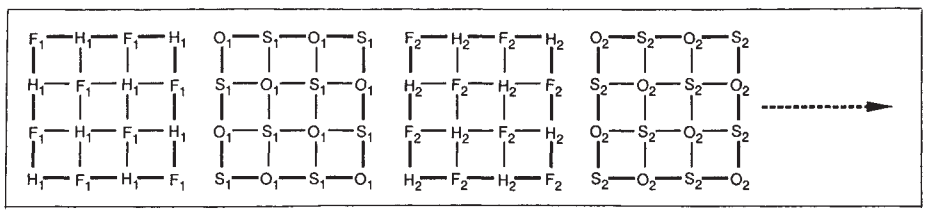

Fig. 1 Planting design. Each 0.64-m^2 array contained either eight naturally pollinated seeds from one of ten females (F) and eight from one of ten hermaphrodites (H), or eight selfed (S) and eight outcrossed (O) seeds from a hermaphrodite, planted at 2-cm depth. Each array containing S and O seeds was planted adjacent to an array containing H seeds from that same individual. Arrays were marked with an aluminium tag at one corner. In each of ten roadside sites, arrays of H and F seeds were replicated five times and arrays of S and O seeds were replicated three times. Because of a shortage of seeds, only three arrays in each site contained naturally pollinated seeds from hermaphrodite number 7, so seeds from another hermaphrodite (number 11) were substituted in the remaining two arrays in each patch. The design called for 1,280 seeds in each of ten sites. Somewhat fewer were planted in sites 6–10 because of seed shortages.

Table 1 Number of surviving plants in August 1987

		Plants											
	Sites	1	2	3	4	5	6	7	8	9	10	11	Total
Self-pollination	2			1		1						—	2
	3											—	0
	4		2	1	1		1		2	1	1	—	9
	5				1	1						—	2
Sub-total		**0**	**2**	**2**	**2**	**2**	**1**	**0**	**2**	**1**	**1**	**—**	**13**
	2		2	3	1	3				3		—	12
	3	2		1	2	1	1					—	7
Cross-pollination	4	2	3	2	4	2	2			2	3	—	20
	5		3		1	1	1					—	6
Sub-total		**4**	**8**	**6**	**8**	**7**	**4**	**0**	**0**	**5**	**3**	**—**	**45**
	2				1	2			2	1		1	7
	3					1					2		3
Hermaphrodite	4	1		4		2			1	1		1	10
	5						1						1
Sub-total		**1**	**0**	**4**	**1**	**5**	**1**	**0**	**3**	**2**	**2**	**2**	**21**
	2	1	1	1	2	3			1		2	—	11
	3		2	1	1			1	3			—	8
Female	4	4	6	9	1	3		3	6		5	—	37
	5				1				1	1		—	3
Sub-total		**5**	**9**	**11**	**5**	**6**	**0**	**4**	**11**	**1**	**7**	**—**	**59**

Only 4 of the original 10 sites produced any survivors.

Table 2 Chi-square values for the association of seedling survival with seed type

Source	d.f.	Self- versus cross-pollination	Hermaphrodite versus female	Self-pollination versus hermaphrodite	Cross-pollination versus female
Intercept	1	486.82†	586.75†	439.26†	738.88†
Seed type	1	15.09†	17.12†	0.11	1.60
Site	3	18.55†	42.26†	15.22*	46.52†

In all comparisons, the association of individual plants with seedling survival and all higher-order associations were not significant and were removed. 0.01 was added to each cell[19].

* $P<0.01$. ‡ $P<0.001$.

one month after pollination[11,12], but may take longer in nature. Naturally pollinated fruits came from unmarked flowers and may have been older than hand pollinated fruits. Weights of naturally pollinated seeds from females and hermaphrodites do not differ (J.R.K., manuscript in preparation).

The following summer (1986), self-, cross- and naturally pollinated seeds of hermaphrodites and naturally pollinated seeds of females, were planted at 10 sites along the highway where the parental plants live (Fig. 1). Planting sites were far enough from existing patches of *C. foetidissima* that no contamination by unplanted seedlings occurred. Seeds were planted between 1 and 10 July, coincident with the onset of summer rains that cue natural germination. Shortly after planting, rains ceased, and there was severe mortality. It was not possible to measure germination and survival independently because seedlings often blew away after drying up, and some seeds, when dug up, were found to have germinated but desiccated before emergence. Only 26 seeds failed to germinate in 1986, but did germinate in 1987, and these were excluded from the analysis. Seedlings were counted on 15–18 August 1986, and again on 19 August 1987. The length of the longest leaf, a predictor of total leaf area ($r^2=0.90$, $P<0.0001$, $n=119$ seedlings), was also recorded.

By mid-August 1986, survival of cross-fertilized seeds of hermaphrodites was greatest, whereas self- or naturally polinated seeds did not differ (means: cross-, 22%; self-, 15%; natural, 15%; $F=5.2$, $P<0.05$). Cross-fertilized seedlings were also larger, but the difference was only marginally significant ($F=3.3$, $P=0.05$). Survival on this date of naturally pollinated seeds from females was not significantly greater than survival of naturally pollinated seeds of hermaphrodites (means: females, 19%; hermaphrodites, 15%; $F=1.79$, not significant), nor were there any detectable size differences.

Only ≈1% of the seeds produced plants that were still alive in August 1987, but differences in survival had become far more pronounced (Tables 1 and 2). Cross-fertilized seeds survived 3.5 (s.e. 0.8) times more frequently than selfed seeds, and seeds of females survived 2.8 (s.e. 0.6) times more frequently than seeds from hermaphrodites (confidence intervals were jackknifed[13]). Survival of naturally pollinated seeds of females and cross-pollinated seeds of hermaphrodites did not differ, nor did the survival of self- and naturally pollinated seeds of hermaphrodites. Seeds from females appear to survive better as a result of the benefits of outcrossing, and this difference in survival is large enough to explain the presence of females at their current frequency[5–8].

Hermaphrodites self-fertilize most of the time and their seeds suffer severe inbreeding depression. Population genetic models that ascribe the negative effects of selfing to lethal (or strongly deleterious) recessive alleles predict that even moderate selfing should rapidly purge a population of this load[14,15]. If this were true for buffalo gourd, the advantage females gain through cross-fertilization would soon decline and females would disappear. Recent models can explain the maintenance of substantial inbreeding depression in highly selfed species if inbreeding depression is modelled as resulting from small deleterious effects of genes at many different loci, or similarly as a quantitative trait, or if the benefits of cross-fertilization are due to overdominance[14,15]. If cross-fertilized seeds survive more than twice as often as self-fertilized seeds, cross-fertilization should be favoured over selfing[16–18]. If inbreeding depression remains severe despite frequent selfing, outcrossing may remain favoured even when plants are commonly forced to self because of pollinator shortages or inefficiencies.

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Electrogenic glutamate uptake in glial cells is activated by intracellular potassium

Boris Barbour, Helen Brew & David Attwell

Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK

Uptake of glutamate into glial cells in the CNS maintains the extracellular glutamate concentration below neurotoxic levels and helps terminate its action as a neurotransmitter[1]. The co-transport of two sodium ions on the glutamate carrier is thought to provide the energy needed to transport glutamate into cells[2,3]. We have shown recently that glutamate uptake can be detected electrically because the excess of Na^+ ions transported with each glutamate anion results in a net current flow into the cell[4]. We took advantage of the control of the environment, both inside and outside the cell, provided by whole-cell patch-clamping and now report that glutamate uptake is activated by intracellular potassium and inhibited by extracellular potassium. Our results indicate that one K^+ ion is transported out of the cell each time a glutamate anion and three Na^+ ions are transported in. A carrier with this stoichiometry can accumulate glutamate against a much greater concentration gradient than a carrier co-transporting one glutamate anion and two Na^+ ions. Pathological rises in extracellular potassium concentration will inhibit glutamate uptake by depolarizing glial cells and by preventing the loss of K^+ from the glutamate carrier. This will facilitate a rise in the extracellular glutamate concentration to neurotoxic levels and contribute to the neuronal death occurring in brain anoxia and ischaemia.

Application of L-glutamate to glial (Müller) cells isolated from the salamander retina evokes an inward current, reflecting electrogenic glutamate uptake[4]. To determine the dependence of this current on internal potassium concentration, we made recordings from voltage-clamped cells, using the whole-cell variant of the patch-clamp technique[5], with pipettes containing different potassium concentrations (KCl replaced by choline chloride). The potassium concentration inside the cell equilibrates with that in the pipette within about two minutes (assessed from the current changes occurring after patch rupture). The specimen records in Fig. 1*a* show that when the potassium concentration in the pipette was high the current was large; when the potassium concentration was zero the current was almost abolished. Replacing all the KCl in the pipette by isotonic sucrose instead of choline chloride produced a similar suppression of the glutamate-evoked current, indicating that the suppression is due to removal of potassium rather than the addition of choline. The small glutamate-evoked current which remained when there was no potassium in the pipette may reflect a low level of potassium remaining in the cell resulting from slow leakage from intracellular organelles.

The dependence of the glutamate-evoked current on pipette potassium concentration approximately fits a Michaelis–Menten curve with an apparent K_m of 15 mM (Fig. 1*b*), implying that the uptake is activated by the binding of one K^+ ion at the inner surface of the cell membrane. The suppression caused by potassium removal was largely due to an effect on the maximum rate of uptake: removal of internal potassium was not associated with any decrease in the apparent affinity of the carrier for glutamate or sodium at the outer face of the cell membrane (Fig. 2). Indeed, potassium removal produced a small increase in the apparent affinity for these substrates (as predicted, see Fig. 2 legend). This indicates that the activation of glutamate uptake by internal potassium is not a result of allosteric alteration by potassium of the structure of the carrier's binding sites for external glutamate or sodium, to promote binding of these substrates.

If intracellular K^+ does not just catalyse glutamate uptake, but is actually transported out of the cell by the carrier, we would expect the glutamate-evoked current to be reduced when the extracellular potassium concentration is high. Raising $[K^+]_o$ (substituted for choline) decreased the current, with a 50% reduction occurring at an extrapolated $[K^+]_o$ of 100 mM (Fig. 3). A similar decrease in current was seen when $[K^+]_o$ was raised by adding KCl to the external solution (making it hypertonic) rather than by substitution for choline chloride. The $[Na^+]_o$-dependence of the current in low and high $[K^+]_o$ indicated that extracellular K^+ does not reduce the current by occupying the Na^+-binding sites on the carrier (see Fig. 3 legend). These results are consistent with the idea that potassium is transported out of the cell by the glutamate uptake carrier, and that a raised $[K^+]_o$ prevents the loss of K^+ from the carrier at the outer surface of the membrane.

We investigated the possibility of chloride co-transport on the glutamate uptake carrier by replacing all the chloride in the external solution by the much larger gluconate anion (six cells) and, in separate experiments, by replacing all the chloride in the pipette solution by gluconate (six cells studied with each pipette solution). In neither case did chloride removal cause a significant change in the magnitude or voltage-dependence of the glutamate-evoked current. Similarly, removal of external calcium or magnesium (or the barium used to block K^+ channels) had no effect on the glutamate-evoked current. Changing the external pH away from 7.3 reduced the current, but did not provide conclusive evidence for transport of H^+ by the carrier (see Fig. 4 legend).

Because glutamate evokes an inward current, there must be a net positive charge transported into the cell during each carrier cycle. Thus, the first-order dependence of the carrier current on internal $[K^+]$ (Fig. 1) and external glutamate concentration (Fig. 2*a*) and the sigmoid dependence on the external sodium concentration (Fig. 2*b*) suggest that the simplest stoichiometry possible for this carrier is one where, for each glutamate anion transported into the cell, one K^+ ion is transported out and three Na^+ ions (or possibly two Na^+ and one H^+) are also transported in (Fig. 4). If only two Na^+ ions were transported there would be no inward current generated by glutamate (assuming that the form of glutamate transported is the form in which over 99% of it exists at physiological *p*H, that is, with one net negative charge). The stoichiometry of $3Na^+:1K^+:1$ glutamate$^-$ was also suggested by radioactive tracing experiments on rat brain vesicles[6] where, however, the membrane potential was not controlled (unlike here) making it difficult to be certain that effects attributed to $[K^+]$ changes were not, in fact, produced by membrane potential changes (because the