

Negative correlation between male allocation and rate of self-fertilization in a hermaphroditic animal

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ABSTRACT Sex-allocation theory predicts that the evolution of increased rates of self-fertilization should be accompanied by decreased allocation to male reproduction (sperm production and broadcast). This prediction has found support in plants but has not previously been tested in animals, which, in contrast to biotically pollinated plants, are free of complications associated with incorporating the costs of attractive structures such as petals. Here we report rates of self-fertilization as well as proportional allocation to male reproductive tissues within populations of the simultaneous hermaphrodite *Utterbackia imbecillis*, a freshwater mussel. Individuals from populations with higher selfing rates devoted a lower proportion of reproductive tissue to sperm production (correlation = -0.99), in support of theory.

The evolutionary theory of sex allocation is particularly amenable to experimental analysis because its predictions are framed in terms of offspring numbers or energy devoted to male and female reproductive processes (1). Much of the theory is directed at understanding sex-allocation patterns within and among taxa based on the principle of optimization by natural selection (2–8). The theory applies both to sex ratios of offspring in dioecious organisms as well as to the proportion of reproductive energy devoted to each sexual function in hermaphrodites. For hermaphrodites practicing some self-fertilization, theory predicts that higher population-level selfing rates should select for lower proportional investment in male function (sperm production) (3, 5, 9–11). This prediction has not previously been tested in animals. Theoretical predictions as well as experimental tests of sex allocation should be more straightforward in animals than in biotically pollinated plants, where the costs and benefits of pollinator attraction must be quantified. Among animals, experimental tests should be most straightforward in groups that do not engage in behaviors, such as egg trading (12, 13), that might be open to cheating. Here we report rates of self-fertilization as well as proportional allocation to male reproductive tissues within populations of the simultaneous hermaphrodite *Utterbackia imbecillis*, a freshwater mussel.

The proportion of reproductive energy optimally devoted to male versus female function in a hermaphrodite should depend on several factors, including the population selfing rate and the shapes of curves relating allocation to sex-specific fertility. When there is no self-fertilization, hermaphrodites are expected to devote equal resources to each function whenever the two fitness-gain curves have the same shape; otherwise, optimal allocation is greater in the sex function for which success increases more rapidly with investment (14). As the mean selfing rate of a population increases, however, an individual's opportunity for fitness gain through sperm broadcast is reduced. The energy spent on sperm broadcast would be

better spent on female processes, such as production of eggs and maturation of young. Increased selfing therefore favors decreased male allocation (11). This is analogous to selection in dioecious organisms for reduced male-progeny sex ratio when local mate competition reduces opportunities for male success (2, 5). Other factors expected to influence optimal allocation have also been studied in theoretical models, including level of inbreeding depression, potential effects of male allocation on an individual's selfing rate, and investment in pollinator attraction (11, 15, 16). The models have found that although these factors may influence the optimal male allocation, the latter nevertheless should decline as higher selfing rates evolve.

MATERIALS AND METHODS

Study Species. *Utterbackia imbecillis* Say (Mollusca: Bivalvia: Unionidae) is a freshwater mussel inhabiting lakes, ponds, canals, rivers, and creeks of eastern North America (17). As in all unionid species, the larval period begins in brood pouches (i.e., interlamellar area of the gills) of the egg-producing parent and continues as a parasite of the gills of fishes. Adults are relatively sessile. Seven of the approximately 300 North American unionid species are simultaneous hermaphrodites (18), and comparative analyses suggest that in each case hermaphroditism is the derived condition (19). Hermaphroditic individuals occasionally can be found in dioecious species (17). Sperm and eggs are released through a common gonoduct, and self-fertilization has been experimentally confirmed in the sole hermaphroditic species tested for this purpose (20).

Selfing Rate. We determined electrophoretic genotypes at nine presumptive protein-encoding loci for individuals of seven populations of *U. imbecillis*. All individuals located at each site (population) were sampled. Starch–gel electrophoresis (21) performed on nongravid gill tissues that had been cleaned and stored at -70°C revealed one or two polymorphic loci per population. Voucher specimens (#253580) have been deposited in the Mollusk Division, Museum of Zoology, University of Michigan. All populations exhibited polymorphism at a maximum of one or two loci. Estimated selfing rates were calculated as $S = 2F/(1 + F)$, where F was the inbreeding coefficient (fixation index; ref. 22). In the case of two polymorphic loci, S was calculated from the average F . The inbreeding coefficient measures deviations from Hardy–Weinberg genotypic proportions expected under random mating: $F = 1 - H_{\text{observed}}/H_{\text{expected}}$, where H is the proportion of heterozygotes. Because types of inbreeding other than selfing also cause nonzero F , S estimated in this way is best considered as the effective rate of self-fertilization within the population. Under any consistent mixture of self-fertilization and outcrossing, F , and therefore S , rapidly achieve equilibrium. F values

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for loci with more than two alleles (which occurred only in the dioecious species) were determined with the program BIOSYS-1 (23). Hypothesis tests were conducted with a nonparametric bootstrap (24) as follows. For each of 10,000 iterations, genotypes of individuals were randomly sampled with replacement N times, where N is the number of individuals in the population, and the expected heterozygosity was calculated from allele frequencies under the hypothesis of random mating ($S = 0$). One-tailed P value for the null hypothesis $S = 0$, versus the alternative $S > 0$, was determined as the proportion of iterations in which the expected heterozygosity exceeded the observed heterozygosity of the original data. One-tailed P value for the null hypothesis $S = 1$, versus the alternative $S < 1$, was determined as the proportion of iterations in which the heterozygosity was zero.

These selfing-rate estimates for *U. imbecillis* were based on the mixed-mating model, in which it is assumed that a fraction S of progeny is produced by self-fertilization and the remainder, by random mating. Nonzero selfing rates were inferred when the proportion of homozygous genotypes exceeded predictions from random mating. Increased homozygosity, however, can also result from biparental inbreeding. Such inbreeding can occur in both hermaphroditic and dioecious species when, for example, a small number of related individuals establish mating populations. To test the possibility that the *U. imbecillis* selfing-rate estimates were strongly affected by types of inbreeding other than self-fertilization, we additionally performed the electrophoretic and statistical analyses on two closely related dioecious species, *U. peggyae* (seven populations) and *U. peninsularis* (six populations) (25).

Sex Allocation. Animals were collected from each of the seven sites, fixed for 12 hr in 10% buffered formalin, and preserved in 70% ethanol. It was impossible to dissect and weigh spermatogenic and oogenic tissues because they interdigitate with the digestive tract and other tissues. The male and female reproductive allocations therefore were determined as follows for each animal. Four equidistant cross-sections through the visceral mass resulted in five tissue wedges that were serially dehydrated in increasing concentrations of propanol, cleared with xylene, and embedded in paraffin (26). Microtome sections ($7 \mu\text{m}$) were mounted on slides treated with Haupt's adhesive, stained with Delafield's hematoxylin to show spermatozoan nuclei, and counterstained with eosin Y to show oogenic tissue. Areas of spermatogenic and oogenic tissues were then measured from each of the five positions by using a dissecting microscope equipped with a video camera and the public-domain image-analysis system NIH-IMAGE (W. Rasband, versions 1.59 and 1.60, available via anonymous file transfer protocol (ftp) from zippy.nimh.nih.gov/pub/nih-image). Microtome slices from the first wedge were taken from the posterior side, whereas those from the other four were from the anterior. Therefore, the areas from wedges one and

two were averaged before summing with the remaining three to obtain a total integrated area for each tissue type. Proportional male allocation for each individual was the sum of the four spermatogenic areas divided by the sum of the four spermatogenic and four oogenic areas.

Statistical Significance of the Correlation. Bootstrap resampling was conducted within populations for both proportional male allocation and rate of self-fertilization, S , as follows. For each of 10,000 bootstrap iterations: mean proportional male allocation for each population was calculated by resampling with replacement N times, where N was the number of individuals sectioned; each population selfing rate was calculated by sampling genotypes with replacement n times, where n was the number of individuals of known genotype (locus chosen with probability 0.5 in populations with two polymorphic loci). S was calculated from F as described earlier. Because deviations from the null hypothesis of no relationship are expected only in one direction (negative), the one-tailed P value was calculated as the proportion of 10,000 regressions for which the slope (or correlation) was greater than or equal to zero.

RESULTS

Estimated rates of self-fertilization, S , ranged from 0.15 to 1.00 for individual populations and from -0.04 to 1.00 for individual loci (Table 1). Three of the seven populations were uninformative with regard to mating system, because S could not be statistically distinguished from either zero or one. The most useful estimates therefore came from the remaining four populations, in which the selfing rate could be distinguished from zero, one, or both (Table 1). Two populations, Oklawaha River ($S = 0.15$) and Dead River Lake ($S = 0.25$), were predominantly outcrossing. Selfing rates were not distinguishable from zero but were significantly less than one. The two remaining populations exhibited high selfing rates that significantly exceeded zero. The value for Suntree Pond ($S = 0.85$) was also less than one, confirming its intermediate rate. Suwannee River Canal ($S = 1.00$) contained only double homozygotes, as expected with two loci and complete selfing.

For the two dioecious species, the mean "selfing" rate among populations was -0.06 for *U. peggyae* and -0.16 for *U. peninsularis* (Table 2). Significance tests confirmed that there was no evidence of biparental inbreeding in either dioecious species, because in each case selfing-rate values were significantly less than one but were indistinguishable from random mating ($S = 0$). In contrast, the average selfing rate among populations for *U. imbecillis* populations was 0.70, an intermediate value statistically distinguishable from both zero and one (Table 2). *U. imbecillis* populations differed significantly in average proportional allocation to spermatogenic tissue (see Table 3; $P < 0.0007$, $n = 37$ individuals, F ratio = 5.4, $df = 6$,

Table 1. Locations and estimated rates of self-fertilization in seven populations of *Utterbackia imbecillis*

Population	N	Locus	Selfing rate	$P <$	
				$H_0: S = 0$	$H_0: S = 1$
Oklawaha River, Marion Co., FL	24	<i>aat</i>	-0.04	0.73	0.36
		<i>pgm</i>	0.30	0.09	0.0001
Dead River Lake, Jackson Co., MS	7	<i>pgi</i>	0.25	0.22	0.02
Little River, Gadsden Co., FL	5	<i>fum</i>	0.69	0.21	0.33
Mill Creek, Jackson Co., IN	12	<i>aat</i>	0.57	0.25	0.12
		<i>pgm</i>	0.86	0.08	0.35
Suntree Pond, Brevard Co., FL	33	<i>aat</i>	0.85	0.003	0.05
Pickering Creek, Chester Co., PA	12	<i>aat</i>	0.86	0.08	0.36
Suwannee River Canal, Dixie Co., FL	34	<i>aat</i>	1.00	0.0001	1.00
		<i>fum</i>	1.00	0.0001	1.00

N , number of individuals; H_0 , null hypothesis.

Table 2. Estimated mean rates of self-fertilization in the hermaphroditic *Utterbackia imbecillis* and its dioecious congeners, *U. peggyae* and *U. peninsularis*

Species	No. of populations	Mean selfing rate	No. of loci	<i>P</i> <	
				<i>H</i> ₀ : <i>S</i> = 0	<i>H</i> ₀ : <i>S</i> = 1
<i>U. imbecillis</i>	7	0.70	10	0.008	0.02
<i>U. peggyae</i>	7	-0.06	18	0.23	0.008
<i>U. peninsularis</i>	6	-0.16	12	0.50	0.02

One-tailed *P* values were determined using Fisher's sign test with one observation per population. *H*₀, null hypothesis.

*R*² = 0.52). Spermatogenic tissue was most abundant in the anterior portion of the visceral mass and declined posteriorly (*P* < 9 × 10⁻⁸, *n* = 170, *F* ratio = 10.8, *df* = 4, *R*² = 0.21). Oogenic tissue was least abundant in the anterior and posterior portions of the visceral mass (*P* < 0.001, *n* = 170, *F* ratio = 4.9, *df* = 4, *R*² = 0.11). As a result, the proportional allocation to spermatogenic tissue declined from 0.55 anteriorly to 0.11 posteriorly (*P* < 10⁻⁹, *n* = 170, *F* ratio = 21.6, *df* = 4, *R*² = 0.34). Individuals within populations did not contribute significantly to overall variation in proportional allocation (general linear model with individuals nested within populations, *P* < 0.13, *n* = 170, *F* ratio = 1.4, *df* = 30, *R*² = 0.39).

Omitting the three populations with uninformative selfing rates, the regression of average proportional male allocation (*a*) on selfing rate (*S*) was *a* = 0.68 - 0.36 *S*, and the Pearson product-moment correlation coefficient was -0.99 (*P* values = 0.021 for both slope and correlation).

DISCUSSION

Previous experimental studies of sex allocation in simultaneously hermaphroditic animals often have focused on mating-group size and competition among individuals to fertilize eggs (local mate competition). Theory predicts that individuals should reduce proportional male allocation as mating-group size decreases and causes local mate competition to increase. The theory is supported by data from fishes (12, 27-29) and a barnacle (30). The selective effects of local mate competition can be explained in several ways (see refs. 5 and 31). To understand the analogy with self-fertilization, consider a reduction in mating-group size. Competition among individuals to fertilize eggs will increase. As a result, the fitness returns per unit of male investment begin decelerating at a lower investment than in a larger group. Local mate competition therefore selects for decreased allocation to male function in hermaphrodites and for lower male-progeny sex ratios in dioecious taxa

Table 3. Mean proportional allocation to spermatogenic tissue in seven populations of *Utterbackia imbecillis*, listed in order of increasing selfing rate

Population	<i>N</i>	Proportional male allocation	
		Mean	(95% CI)
Oklawaha River	4	0.61	0.59-0.64
Dead River Lake	6	0.60	0.40-0.77
Little River	5	0.19	0.09-0.28
Mill Creek	6	0.24	0.16-0.32
Suntree Pond	6	0.40	0.34-0.49
Pickering Creek	6	0.35	0.19-0.51
Suwannee River Canal	4	0.30	0.24-0.36

For each individual, total spermatogenic and oogenic areas were determined by summing over four equidistant cross-sections through the visceral mass. Confidence intervals (CI) were determined as ordered observations 250 and 9,750 from 10,000 bootstrap random samples (with replacement) of *N* individuals for each population. *N*, number of individuals.

(2-5, 12, 31-33). Similarly, as self-fertilization increases, the pool of eggs available to be fertilized by outcrossed sperm diminishes, and each sperm, or unit of male investment, is competing for fewer available eggs (5, 11).

The present study found that mussels in populations with higher rates of self-fertilization devoted a lower proportion of reproductive tissue to sperm production. Male allocation ranged from about 0.3 to 0.6. In *Rivulus marmoratus* (Cyprinodontidae), a simultaneously hermaphroditic fish with a very high natural selfing rate, Harrington (34) reported male allocation to be approximately 0.03, one-tenth the value found in the most highly selfing mussel population. The correlation between selfing rate and proportional male investment previously has been investigated only in plants, which display great evolutionary lability in mating systems. Among unrelated angiosperm taxa, it has long been appreciated that highly selfing species, as often judged by floral-morphological characteristics, produce less pollen than highly outcrossing species (35-37). Lloyd (38) found that pollen production in species of *Cotula* (Asteraceae) judged more likely to be outcrossers was lower than in species judged to be more highly selfing. Among nine species of wind-pollinated herbs, the effort (mass) devoted to anthers relative to seeds was lower in self-compatible, and therefore potentially self-fertilizing, species than in self-incompatible (*n* = 1) or dioecious species (*n* = 2) (39).

Correlations between selfing rate and sex allocation can be measured only when the mating system is quantified. Genetic markers can be used to estimate population selfing rates either from inbreeding coefficients (22), as in the present study, or by analysis of progeny genotypes (40, 41). Furthermore, tests are best conducted between closely related species or populations within species, so that unmeasured factors do not confound the correlation. There are very few studies meeting both criteria, and all have found a negative relationship (42). With selfing rates ranging from 0.04 to 0.85 among seven populations of *Gilia achilleifolia* (Polemoniaceae), an insect-pollinated annual, Schoen (43) found the correlation between proportional male allocation and selfing rate to be approximately -0.99, similar to the value found in the present study. A similar negative correlation (approximately -0.9) occurred among six populations of *Eichhornia paniculata* (Pontederiaceae), where selfing rates ranged from 0 to 0.9 (44). The correlation was approximately -0.5 among eight closely related species of *Mimulus* (section *Simiolus*, Scrophulariaceae), among which selfing rates ranged from 0.31 to 0.84 (45). The *Mimulus* allocations were measured at flowering and therefore omitted seed production, which is the major portion of female allocation. It seems likely that the absolute value of the correlation would be somewhat increased if measured at the fruiting stage, as was found by comparing the two measures in *Eichhornia* (44). A negative but statistically nonsignificant relation was found among Jamaican populations of *Turnera ulmifolia* (Turneraceae), where selfing rates ranged from 0 to 0.31 (46). In the only study of a wind-pollinated plant, Charnov (16) investigated 31 varieties of wild rice, *Oryza perennis* (Poaceae), and found a correlation of -0.84 between selfing rate and the ratio of anther mass to seed mass, a measure of male allocation slightly different from that used in the other studies.

Our method assumed that the relevant components of reproductive allocation have been measured. Brooding may represent an unmeasured female cost, given that some extra tissue is devoted to gills and that larvae may reduce food intake of the parent. We were, however, concerned with the decline of male allocation with selfing rate, rather than absolute measures of allocation. It seems unlikely that incorporating costs of brooding would have much effect on this relationship. Our method furthermore assumed that the cost per tissue area remained constant at all levels of allocation. Unequal true costs per area for male and female tissues would again change the absolute allocations but not the pattern of decline. Gonads

might also mature at slightly different rates within seasons, which would introduce some error into the estimates of sex allocation (17). Such error should not be systematic, because our collections occurred throughout the reproductive season.

The mussel populations showed great variability in mating system, from nearly complete outcrossing to complete selfing. Evolution of rates of self-fertilization depends on the balance of several ecological and genetic factors, as well as on phylogenetic history. For example, self-fertilization should be advantageous when the probability of successful outcrossing is low, as in colonization events (47). Self-fertilization furthermore transmits two gene copies to each offspring, as compared with one through outcrossing. This genetic transmission advantage automatically selects for increased selfing rates unless checked by other factors, such as lowered fitness of progeny from selfing (inbreeding depression) or reduced success through sperm broadcast (sperm or pollen discounting) (48). In addition, mutations causing large increases in the selfing rate can increase in frequency regardless of the level of inbreeding depression, because individuals bearing such mutations will form a subpopulation that is largely genetically isolated (49).

Previous reports on *U. imbecillis* suggested that low male investment is characteristic of high-density, central-range populations of lakes and canals, whereas high male investment is characteristic of lower-density, peripheral populations of creeks and rivers (17). Those reports, however, measured the ratio of male to female areas, rather than male to total. They furthermore failed to determine areas throughout the visceral mass, reporting this to be relatively constant, in disagreement with the present results.

The generality of sex-allocation theory will be determined only by examining diverse taxa, and current sex-allocation theory is framed in terms that should apply equally to plants and animals. Hermaphroditism and self-fertilization are common among the vascular plants (50) but proportionally rarer among animal species. Nevertheless, several animal phyla, such as mollusks, include both dioecious and hermaphroditic groups (51, 52) in which it should prove profitable to investigate the evolutionary forces acting on rate of self-fertilization. Testing sex-allocation theory in plants often involves measuring investment in attractive structures and then either assigning a fraction of the investment to male and female function (53) or determining the effects of attraction on success through male function, female function, and self-fertilization (15). Animals, in contrast, do not attract or pay biotic agents to transfer gametes and should therefore prove especially useful for straightforward tests of many predictions of sex-allocation theory. These tests ideally will be not made among higher taxa but rather among closely related populations or species, where it is most likely that the selective forces are currently operating (ref. 3, p. 123).

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- Orzack, S. H. (1993) in *Evolution and Diversity of Sex Ratio in Insects and Mites*, eds. Wrensch, D. L. & Ebbert, M. A. (Chapman & Hall, New York), pp. 477–511.
- Hamilton, W. D. (1967) *Science* **156**, 477–487.
- Williams, G. C. (1975) *Sex and Evolution* (Princeton Univ. Press, Princeton, NJ).
- Maynard Smith, J. (1978) *The Evolution of Sex* (Cambridge Univ. Press, Cambridge, UK).
- Charnov, E. L. (1982) *The Theory of Sex Allocation* (Princeton Univ. Press, Princeton, NJ).
- Leigh, E. G., Jr., Herre, E. A. & Fischer, E. A. (1985) *Experientia* **41**, 1265–1276.
- Karlin, S. & Lessard, S. (1986) *Theoretical Studies on Sex Ratio Evolution* (Princeton Univ. Press, Princeton, NJ).
- Frank, S. A. (1987) *Theor. Pop. Biol.* **31**, 47–74.
- Maynard Smith, J. (1971) in *Group Selection*, ed. Williams, G. C. (Aldine-Atherton, Chicago), pp. 163–175.
- Lloyd, D. G. (1979) *New Zealand J. Bot.* **17**, 595–606.
- Charlesworth, D. & Charlesworth, B. (1981) *Biol. J. Linn. Soc.* **15**, 57–74.
- Fischer, E. A. (1981) *Am. Nat.* **117**, 64–82.
- Petersen, C. W. (1995) *Environ. Biol. Fishes* **43**, 351–361.
- Lloyd, D. G. (1984) in *Perspectives on Plant Population Ecology*, eds. Dirzo, R. & Sarukhan, J. (Sinauer, Sunderland, MA), pp. 277–300.
- Charlesworth, D. & Charlesworth, B. (1987) *Evolution* **41**, 948–968.
- Charnov, E. L. (1987) *Evol. Ecol.* **1**, 30–36.
- Kat, P. W. (1983) *J. Zool.* **201**, 395–416.
- Burch, J. B. (1975) *Freshwater Uniacean Clams (Mollusca: Pelecypoda) of North America* (Malacological Publications, Hamburg, MI).
- Hoeh, W. R., Frazer, K. S., Naranjo-Garcia, E. & Trdan, R. J. (1995) *Malacol. Rev.* **28**, 25–42.
- Bloomer, H. H. (1943) *Proc. Malacol. Soc. London* **25**, 192–200.
- Shaw, C. R. & Prasad, R. (1970) *Biochem. Genet.* **4**, 297–320.
- Wright, S. (1969) *Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies* (Univ. of Chicago Press, Chicago).
- Swofford, D. L. & Selander, R. B. (1981) *BIOSYS-1: A Computer Program for the Analysis of Allelic Variation in Genetics* (Univ. of Illinois, Urbana).
- Efron, B. & Tibshirani, R. J. (1993) *An Introduction to the Bootstrap* (Chapman & Hall, New York).
- Bogan, A. E. & Hoeh, W. R. (1995) *Walkerana* **7**, 275–287.
- Humason, G. L. (1972) *Animal Tissue Techniques* (Freeman, San Francisco).
- Fischer, E. A. (1984) *Am. Nat.* **124**, 590–596.
- Fischer, E. A. & Petersen, C. W. (1987) *Bioscience* **37**, 482–489.
- Petersen, C. W. (1991) *Am. Nat.* **138**, 650–667.
- Raimondi, P. T. & Martin, J. E. (1991) *Am. Nat.* **138**, 1206–1217.
- Bulmer, M. G. (1994) *Theoretical Evolutionary Ecology* (Sinauer, Sunderland, MA).
- Charnov, E. L. (1996) *Evol. Ecol.* **10**, 457–462.
- Wrensch, D. L. & Ebbert, M. A., eds. (1993) *Evolution and Diversity of Sex Ratio in Insects and Mites* (Chapman & Hall, New York), p. 630.
- Harrington, R. W. (1971) *Copeia* **3**, 389–432.
- Ornduff, R. (1969) *Taxon* **18**, 121–133.
- Lovett Doust, J. & Cavers, P. B. (1982) *Can. J. Bot.* **60**, 2530–2534.
- Bertin, R. I. (1987) in *Reproductive Strategies of Plants*, eds. Lovett Doust, J. & Lovett Doust, L. (Oxford Univ. Press, Oxford, UK).
- Lloyd, D. G. (1972) *New Phytol.* **71**, 1181–1194.
- Lemen, C. (1980) *Oecologia* **45**, 156–159.
- Schoen, D. J. & Clegg, M. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5258–5262.
- Ritland, K. (1986) *Biometrics* **42**, 25–43.
- Brunet, J. (1992) *Trends Ecol. Evol.* **7**, 79–84.
- Schoen, D. J. (1982) *Oecologia* **53**, 255–257.
- Morgan, M. T. & Barrett, S. C. H. (1989) *J. Evol. Biol.* **2**, 183–203.
- Ritland, C. & Ritland, K. (1989) *Am. J. Bot.* **76**, 1731–1739.
- Belaoussoff, S. & Shore, J. S. (1995) *Evolution* **49**, 545–556.
- Baker, H. G. (1955) *Evolution* **9**, 347–348.
- Lloyd, D. G. (1992) *Int. J. Plant Sci.* **153**, 370–380.
- Lande, R. & Schemske, D. W. (1985) *Evolution* **39**, 24–40.
- Yampolsky, C. & Yampolsky, H. (1922) *Bibl. Genet.* **3**, 1–62.
- Ghiselin, M. T. (1969) *Q. Rev. Biol.* **44**, 189–208.
- Jarne, P., Vianey-Liaud, M. & Delay, B. (1993) *Biol. J. Linn. Soc.* **49**, 99–125.
- Goldman, D. A. & Willson, M. F. (1986) *Bot. Rev.* **52**, 157–194.