Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants

D. Wiens[1], C.L. Calvin[2], C.A. Wilson[2], C.I. Davern[1], D. Frank[3], and S.R. Seavey[4]

[1] Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
[2] Departments of Biology and Chemistry, Portland State University, Portland, OR 97207, USA
[3] Department of Biology, Syracuse University, Syracuse, NY 13210 USA
[4] Department of Biology, Lewis and Clark College, Portland, OR 97219, USA

Summary. Reproductive success is divided into two phases: preemergent (the number of viable seeds that enter the ambient environment) and postemergent (the percentage of progeny that survive to reproduce). We studied preemergent reproductive success (PERS) in flowering plants by measuring the fruit/flower (Fr/Fl) ratio and the seed/ovule (S/O) ratio in a number of species of outcrossing and inbreeding plants, where PERS = the product of (Fr/Fl) and (S/O). In order to determine the influence of the ambient environment (including resource availability) we studied pairs of outcrossing and inbreeding species occurring in the same habitat. Among outcrossing species PERS averaged about 22%, whereas in inbreeding species the average was approximately 90%. The progeny/zygote (P/Z) ratio was studied in hand-pollinated populations in *Epilobium angustifolium* (a strongly outcrossing species) from populations in Oregon and Utah, by direct observation of embryogenesis at two-day intervals throughout the course of seed development. The P/Z ratio in both populations averaged near 30%, and the developing embryos showed a surprising array of abnormalities that resulted in embryo death. During early development >95% of the ovules had normally developing globular embryos, but beginning with differentiation (cotyledon formation) about 70% of the original globular embryos aborted during the course of embryogenesis and seed development. The clustering of developmental lethals during periods of major differentiation events parallels the animal model of development. We found little evidence that PERS was limited by the ambient environment (including resource availability), pollination, or factors associated with the inbreeding habit. Instead, PERS was found to be inextricably linked to outcrossing plants, whose breeding systems promote genetic variability. The high incidence of developmental lethals in *E. angustifolium* and the resulting low P/Z ratio (ca. 30%) is attributed to genetic load (any lethal mutation or allelic combination) possibly working in combination with developmental selection (interovarian competition among genetically diverse embryos). Examples of maternally controlled, fixed patterns of ovule abortion with respect to position or number are discussed. However, we found no need to employ "female choice" as a hypothesis to explain our results for the extensive, seemingly random patterns of embryo abortion in *E. angustifolium* and other outcrossing species. A more parsimonious, mechanistic explanation based on genetic load-developmental selection is sufficient to account for the differential survivorship of embryos. Likewise, the traditional concept of a positive growth regulator feedback system based on the number of surviving ovules in an ovary can account for subsequent fruit survivorship.

Key words: Epilobium – Fruit/flower ratio – Seed/ovule ratio – Progeny/Zygote ratio

We define reproductive success as the number of eggs surviving to reproduce successfully. This constitutes a central issue in biology. Reproductive success is not only *the* measure of Darwinian fitness, but is also the major focus of agriculture and a critical aspect of human reproduction. We divide reproductive success into preemergent and postemergent phases. Preemergent reproductive success is the number of eggs that complete development and survive to enter the ambient environment; presumably it reflects selection on the embryo phenotype during development. In seed plants it is measured by the number of ovules that mature into viable seeds released from the maternal parent. Postemergent reproductive success is the percentage of progeny that survive to reproduce following their release into the ambient environment. In seed plants, postemergent reproductive success represents the stages in the life cycle from seed germination to reproductive module production and fertilization; among animals, it constitutes successful maturation and ultimate mating of the newborn young or hatchlings in vertebrates and invertebrates. Typically, it has been this postemergent phase of the life cycle of both plants and animals that has been studied in selection experiments, while the preemergent phase has been largely ignored.

The object of this study was to analyze preemergent reproductive success in flowering plants as affected by (1) resource availability and (2) pollination.

Plants are exceptionally good experimental subjects for the study of reproductive success because they: (1) are sessile; (2) are often widely distributed and abundant; (3) possess a diversity of breeding systems uncommon in other organisms; and, (4) typically produce many reproductive modules (flowers and fruits) each bearing a relatively large number of progeny (seeds) that are easily analyzed without sacrificing the organism.

Table 1. Mature fruit/flower ratios, seed/ovule ratios, and preemergent reproductive success for outcrossing and inbreeding co-occurring species pairs, co-occurring congeneric species pair, and other inbreeding and outcrossing species of *Epilobium*

	Fr/Fl ratio	N	S.D.	S/O ratio	N	S.D.	Life history[d]	PERS
Outcrossing[a] co-occurring species	0.459	9 (species)	19.0	0.480	9	9.8	PH	0.221
Inbreeding[a] co-occurring species	0.927	9	6.0	0.918	9	7.5	A	0.852
Outcrossing congener (*E. angustifolium*)	0.596	10 (pop)	7.4	0.372	10	17.5	PH	0.223
Inbreeding congener (*E. ciliatum*)	0.984	10	2.7	0.953	10	2.7	PH	0.938
All outcrossing[b] species of *Epilobium*				0.451	3	10.5	PH	
All inbreeding[c] species *Epilobium*				0.907	6	5.0	Ph&A	

[a] *Agastache urticifolia* (outcrosser) Fr/Fl=25.8% (N=78), S/O=55.9% (N=17), PERS=17.2%; *Descurainia richardsonii* (inbreeder) Fr/Fl=97.8% (N=509), S/O=94.1% (N=15), PERS=92.0%; *Allium acuminatum* 67.6% (188), 45.0% (10), 30.4% *Veronica biloba* 91.1% (145), 94.0% (15), 85.6%; *Hedysarum occidentale* 52.9% (287), 52.8% (54), 27.9%; *Lepidium hirsutum* 90.0% (571), 88.1% (15), 79.3%; *Lupinus argenteus* 17.1% (328), 48.3% (15), 8.3%; *Polygonum douglasii* 100.0% (112), 100.0% (112), 100%; *Phacelia hastata* 65.2% (161), 27.4% (21), 17.7%; *Gayophytum ramosissimum* 94.4% (54), 91.0% (15), 85.9%; *Penstemon cyananthus* 34.2% (149), 42.5% (6), 14.5%; *Epilobium paniculatum* 93.8% (64), 94.6% (165), 88.6%; *Polemonium foliosissimum* 70.8% (89), 59.5% (10), 42.1%; *Verbena bracteatum* 97.2% (108), 100.0% (15), 97.2%; *Scrophularia californica* 44.1% (913), 56.7% (7), 25.0%; *Barbarea orthoceras* 80.0% (280), 98.0% (15), 71.2%; *Smilacina stellata* 35.0% (100), 43.9% (11), 15.4%; *Androsace septentrionalis* 89.6% (154), 75.2% (15), 67.3%

[b] Outcrossing species of *Epilobium* (S/O ratios only). *E. angustifolium:* 58.7% Ouray, Colorado; 49.9% Alta, Utah in 1984 – 34.1% in 1985; 35.8% Onion Valley (Inyo Co.), California in 1984 – 31.4% in 1985; 43.6% Portland, Oregon. *E. canum:* 56.6% Brighton, Utah. *E. luteum:* 50.8% Mt. Hood, Oregon

[c] Inbreeding species of *Epilobium* (S/O ratios only). *E. alpinum:* 84.4%, 92.1%, 77.8%, 89.0% Henrys Fk. Lake (Summit Co.), Utah. *E. ciliatum:* 97.7% Alta, Utah; 95.7% Onion Valley (Inyo Co.), California. *E. clavatum:* 85.8% Trail Ridge Rd., Colorado. *E. hornemannii:* 85.0% Cameron Pass, Colorado. *E. lactiflorum:* 91.8% Gould, 95.1% Cameron Pass, Colorado. *E. paniculatum:* 94.7% Granby, Colorado; 85% Alta, 94.6% Brighton, 91.0% Millcreek Cyn., 96.4% Red Butte Cyn., Utah; 94.3% Portland, Oregon

[d] *PH* perennial herb; *A* annual

Materials and methods

Preemergent reproductive success (PERS) is affected by the progeny/zygote (P/Z) ratio in all organisms. In plants and other groups with multiple reproductive modules, however, the problem is slightly complicated by the abortion of whole modules. Thus in angiosperms, flowers (Fl) are the immature modules, and fruits (Fr) are their matured derivatives; therefore PERS the product of (Fr/Fl) and (P/Z). If data on zygote formation are not available, as is often the case in plant studies, the seed/ovule (S/O) ratio can be used as an estimate for the P/Z ratio. The S/O ratio, however, tends to overestimate PERS by the lack of inclusion of germination data for seeds and compensates by failing to distinguish between lack of fertilization and subsequent early abortion of zygotes. These limitations are not viewed as serious detriments to the aims of this study.

To determine if resource availability or genetic load affected S/O ratios, we studied species occurring in the same habitat, but characterized by either autogamous or outcrossing breeding systems. This methodology assumes that inbreeding species are essentially homozygous genetically (we recognize that low levels of heterozygosity may be retained) and as a result are largely free of lethal alleles. Conversely, outcrossing plant populations possess a heterozygous genetic system capable of carrying recessive deleterious alleles in equilibrium, as well as generating recombinational load (Wiens 1984). Mutational load, especially in long-lived perennials, may be exhibited by both groups of plants, but it should be greater among outcrossers because of the mutational load bearing capacity of the outcrosser's gene pool (Muller 1964).

We determined PERS in three experimental groups: (1) co-occurring species pairs, i.e., two phyletically unrelated species growing in the same habitat within at least a meter of each other. One of these was an inbreeding annual (usually weedy), and the other, an outcrossing, indigenous, perennial herb (such combinations are found typically along the margins of disturbed areas); we studied 9 co-occurring species pairs.

(2) A co-occurring congeneric pair, i.e., two species of the same genus (*Epilobium*, Onagraceae), one an inbreeder (*E. ciliatum*), the other strongly outcrossing (*E. angustifolium*); both are perennial herbs and shared the same habitat, growing within decimeters of each other; 10 different populations of this co-occurring congeneric pair were studied. For this group we also measured plant water potentials and soil water percentage. A minimum of 10 inflorescences and 10 fruits were used to determine PERS in each population of these two experimental groups; both studies were conducted near Brighton in the Wasatch Mountains, Utah.

(3) Flowers from two populations of *E. angustifolium* were hand-pollinated in the field to insure sufficient pollination and very high pollen to ovule ratios. The P/Z ratios were determined by direct observation of cleared embryos (Herr 1974), a technique that proved immensely valuable in this study. One population was near Brighton, Utah (el. ca. 2,400 m), the other was from Portland, Oregon (near sea level). Embryo survivorship in both populations was analyzed at three and five days post pollination and at two day intervals from 10 days to 26 days post pollination. By the latter date the fruits appeared mature and ready to dehisce. All data points were based on the analysis of two to four fruits, except the last analysis which utilized
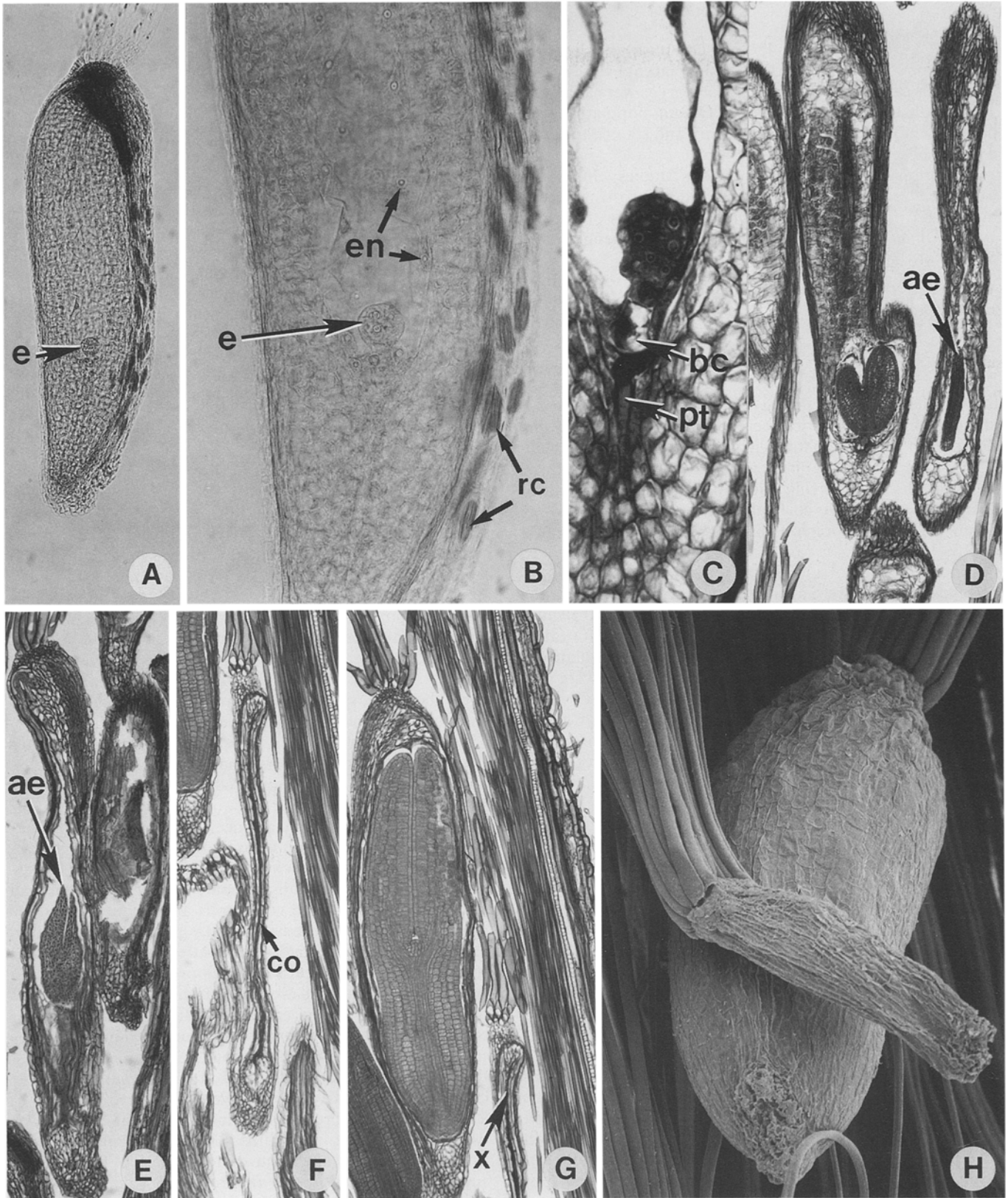
Fig. 1 A–H. Cleared ovules (**A, B**), longitudinally sectioned ovules (**C–G**), and mature seeds (**H**) of *Epilobium angustifolium* as seen using bright field (**A–G**) and scanning electron microscopy (**H**). The micropylar end of the ovule is to the lower side in each photograph. **A–C**, ten days post pollination (Utah): **A** whole ovule with globular embryo (*e*), ×40; **B** higher resolution view of ovule seen in **A** to show embryo, endosperm nuclei (*en*), and raphid crystals (*rc*), ×100; **C** embryo showing short suspensor with enlarged basal cell (*bc*), and remnants of pollen tube (*pt*), ×160; **D** normal and aborting embryo (*ae*) at ten days post pollination (Oregon), ×40; **E–G** eighteen days post pollination (Oregon): **E** aborting embryo ×40; **F** collapsed ovule (*co*) with necrotic embryo ×40; **G** full size embryo, portion of collapsed ovule at *"X"* is the same ovule as seen in **F**, ×40; **H** mature seeds (The lower ovule is large and plump and contains a viable embryo. In the smaller, collapsed ovule the embryo has aborted. In both naturally-pollinated and hand-pollinated flowers these two seed types occur in approximately equal numbers), ×120

10 fruits. A minimum of 40 and up to several hundred ovules from the central portion of the placenta were scored from each capsule. A total of 2,981 embryos were analyzed. The middle third of fruits from the Oregon population were paraffin embedded, sectioned (12 μm), and stained for more detailed observations.

To supplement the three previous studies, S/O ratios were analyzed in other inbreeding and outcrossing species of *Epilobium*, as well as additional populations of both *E. angustifolium* and *E. ciliatum* from different geographical regions of the western United States.

Plants obviously suffering from stress due to the physical environment, predators, or pathogens were excluded from the sample.

Results

In both the nine co-occurring species pairs and the ten populations of the congeneric species pairs, the outcrossing species in both groups had low, virtually identical PERS values (0.221). The inbreeding species exhibited high PERS values, 0.852 for the co-occurring pairs and 0.938 for the congeneric pair (Table 1). No correlation, however, was found between geographical region, habitat, or soil moisture and PERS.

The S/O ratios of *Epilobium* from widely disparate geographical regions, were also consistent with those from the co-occurring and congeneric species pairs, i.e., 0.50 and 0.90, respectively (Table 1). Previous studies of S/O ratios of a large number of species growing in natural populations, and a few cultured under greenhouse conditions, were also consistent with these data (Wiens 1984). The S/O ratios of *E. angustifolium* and *E. ciliatum* from Utah and California were also statistically consistent over two seasons.

The 10 populations of the congeneric pair did not exhibit water potentials indicative of moisture stress (*E. angustifolium* c̄ 1.2 MPa, *E. ciliatum* c̄ 1.4 MPa) even though PERS was low for *E. angustifolium* (0.22). For each of the 10 sites, there was no significant difference between the water content (by wt.) of the soils in which both the outcrossing species (*E. angustifolium*) and the inbreeding species (*E. ciliatum*) were growing. An annual inbreeding species (*E. paniculatum*) has S/O ratios averaging 0.927 from populations in Colorado, Utah, and Oregon. These are also similar to those for all inbreeding, perennial species of *Epilobium*, indicating once again that the S/O ratio is a function of the breeding system, and not directly related to life history, even though most annuals are inbreeders and perennials outcrossers (Wiens 1984).

The S/O ratios of naturally, open pollinated populations of *E. angustifolium* (Table 1) were, in fact, higher than the P/Z ratios of the hand-pollinated populations that exhibited 97% zygote formation (Fig. 2). Apparently, pollination does not generally limit PERS in this species, although it might contribute to whole fruit abortion – a subject not pursued in this study.

In the populations of *E. angustifolium* from Oregon and Utah in which the flowers were hand-pollinated and embryogenesis observed directly from cleared ovules, development to the globular phase (Figs. 1A–C) occurred in 97% of ovules (Fig. 2). More detailed observations from sectioned and stained material showed that at the globular phase, which in Oregon occurs approximately eight days after pollination, each embryo has a short suspensor with
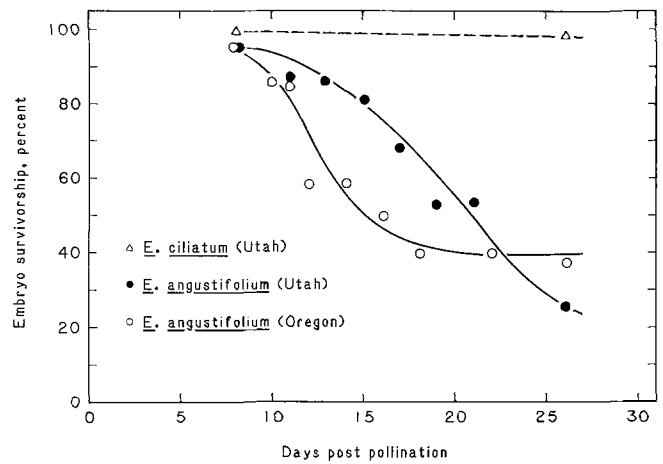

Fig. 2. Percent of embryos surviving over time for *E. ciliatum* from Utah and *E. angustifolium* from Oregon and Utah. *E. ciliatum* is an autogamous, inbreeding perennial, whereas *E. angustifolium* is a strongly outcrossing perennial

an enlarged basal cell, and most embryos have a clearly delimited protoderm (Fig. 1 C). But just ten days later, when successful embryos typically have reached full size, approximately 65% of the initiated embryos have aborted (Fig. 2). Ovules with embryos of full size (Fig. 1 G) are readily distinguished from those with failed embryos (Fig. 1 F) and seeds of the former are large and plump at maturity, whereas the latter are smaller and collapsed (Fig. 1 H). Similar results were also obtained from the Utah population, however, the temporal sequence varied and the final progeny/zygote (P/Z) ratio was lower (Fig. 2).

Embryo abortion in *E. angustifolium* occurs at various developmental stages and is manifest in several ways. A small percentage of abortion occurs prior to the mid-globular stage. These embryos show irregular divisions in the suspensor and/or embryo proper. A somewhat larger percentage of embryos continue to grow as ever enlarging globules and never initiate cotyledons. The cells in these enlarged globules eventually become highly vacuolate and then necrose. In others, cotyledons are initiated but these are malformed, some being sharply pointed, others club-shaped. Still other embryos abort after the cotyledons and hypocotyl/root axis are well developed. A slowing of embryo growth is often a first symptom of impending abortion. Nearly all failing embryos shift position within the embryo sac, either becoming tilted or moving excessively toward the chalazal end, or both. At maturity many of the developing seeds collapse. Careful examination of these collapsed seeds nearly always reveals the presence of a pollen tube and/or necrotic tissue, indicating that these ovules were fertilized, but later failed. The endosperm often continues to grow for some time after the death of the embryo, suggesting that endosperm failure is not a common cause of embryo loss, at least in *E. angustifolium*.

Discussion

There is nothing in our results to indicate that either resource availability or pollination limited seed set in either the co-occurring species pairs or the congeneric species pair. Our overall values for S/O ratios in *E. angustifolium*, however, are considerably lower (0.37) than those obtained by

Flint (1980), who grew both tetraploid (0.70) and diploid (0.46) plants of this species under controlled greenhouse conditions. Plants from both Utah and Oregon are within the distributional limits of the tetraploid (Mosquin 1966), although our S/O ratios are closer to those of the diploid (chromosome number was not determined).

Our data clearly show that a high incidence of developmental abnormalities and embryonic death consistently occurs in the strongly outcrossing species, E. angustifolium, and are virtually absent in its inbreeding congener, E. ciliatum, even when they occupy the same habitat. The evidence from Epilobium, as well as the co-occurring species pairs, shows that increased embryonic and whole fruit abortion is inextricably linked to plants with breeding systems whose gene pools harbor more genetic variation. This was also demonstrated most convincingly in outcrossing and inbreeding populations in Gilia achilleifolia where both seed yield/fruit and fruit set decreased with increasing rates of outcrossing, as well as increased genetic heterogeneity as measured by allozyme variation (Schoen 1981, 1982).

Our data provide little support for the proposition that among outcrossing plants, the S/O ratio is resource or pollen limited. The inbreeding species with which the outcrossers grow (often with their roots intertwined) should act as effective controls on this point. We believe the concept of genetic load (Muller 1950) is sufficient to explain the low values of PERS we found among outcrossers (we use the term in the broad sense to include all deleterious mutations and allelic combinations). We see no necessity to invoke the concept of "female choice" as a separate and distinct mechanism to account for the patterns of PERS we report. In agreement with Buchholz (1922), we also employ his idea of developmental selection, i.e., intraovarian competition among genetically diverse embryos, as a mechanism leading to the abortion of less vigorous embryos.

"Female choice" or maternal control is currently the subject of intensive interest in terms of embryo and whole fruit abortion. The genetic load-developmental selection hypothesis and the "female choice" hypothesis are both based on the premise of genetic variability among the developing embryos, but they are separable on the basis of the mechanism by which embryo abortion occurs. The genetic load-developmental selection hypothesis is a mechanistic explanation. "Female choice", however, connotes an additional but unspecified mode of operation that is presumably also the product of natural selection, rather than (as we suspect) some corollary to the way in which plants have evolved to establish and regulate their maternal-progeny interactions.

Early embryonic development in animals is maternally controlled, since the protein necessary for cleavage is coded by RNA transcribed from the maternal nucleus (Browder 1984). Thus, in animals, the zygotic genome is activated at the onset of major differentiation events, i.e., blastula formation or more commonly gastrulation; it is at these stages of embryogeny that a high incidence of developmental lethals is expressed (Hadorn 1961), presumably because the lethals are not exposed until the zygotic genome begins to function. Many regulatory genes must be involved in embryo differentiation, that are perhaps not otherwise activated, and many structural genes are first utilized during this period. The expression of lethals in such genes would thus be limited to this brief, but critical, stage of the life cycle. Seedling development in plants and molting in insects

might be additional phases where the consequences of such lethals might cluster.

The animal model of development could explain several critical aspects of embryogenesis in E. angustifolium, viz., the high percentage of embryos surviving through the globular phase, and the initiation of high abortion rates associated with the initiation of differentiation (cotyledon formation).

Although there is little literature on the subject, it is highly likely that a similar pattern occurs in plants (Sang 1984). During early embryogeny, the embryo proper is an enlarging, spherical mass of cells. As the embryo continues its development, major differentiation events are initiated (Esau 1977), including: 1) the blocking out of tissue systems; 2) the formation of lateral appendages (cotyledons and foliage leaves); 3) a change from axial to bilateral symmetry; 4) the establishment of organized shoot and root meristems; and, 5) the synthesis of seed storage proteins (to say nothing of the metabolic proteins that are concerned with normal cellular activities, including the synthesis of storage proteins). In some cases, mature tissues, such as conducting elements of xylem and/or phloem, may also differentiate as embryos approach maturity.

There is a suggestion of two steps in the sigmoid curve in Fig. 2. Two distinct periods of increased abortion are known in some fruit crops (e.g., peaches) and correlate with increased rates of whole fruit abortion at these times (Bradbury 1929). For additional examples and discussion, see Stephenson (1981).

Genetic load. Our embryological data strongly favor the involvement of this factor. The distribution of abortion throughout the entire course of embryogenesis, following the globular stage, strongly supports the proposition that they are the result of developmental lethals. Developmental lethals have been the subject of intensive research over the last several decades, particularly in *Drosophila,* and there is now an extensive literature on the subject. Nothing in our observations suggest that the abortion of variously aberrant embryos observed in E. angustifolium are the result of causes other than developmental lethals and competitive loss of weaker embryos. The abnormal morphological patterns or malfunctioning cell processes leading to embryo death in E. angustifolium, do not differ in any fundamental way from the action of developmental lethals in animals, or those aberrations accepted as developmental lethals in *Arabidopsis* and maize (Meinke and Sussex 1979, Meinke 1985, Marsden and Meinke 1985, Sheridan and Neuffer 1982). Likewise the temporal sequences of development in E. angustifolium, i.e., high percentages of embryo development to the globular phase, followed by high abortion rates at differentiation when the zygotic genome is activated, all conform to the animal model of development discussed previously, and support the genetic load hypothesis.

Developmental selection among the genets of long-lived perennials has been identified by Klekowski et al. (1985) as a means by which a plant deals with the accumulation of genetic load by somatic mutations in the meristems.

Endosperm dysfunction can also result in embryo death, but we found little evidence of this in E. angustifolium. In Epilobium, both the endosperm and embryo are diploid (Johri 1963), and are thus presumably identical, both quantitatively and qualitatively. Conifers have no endosperm but Sorensen (1969) has reported S/O ratios in Douglas

fir (<0.50) that are generally similar to those of flowering plants.

The importance of the internal environment might be tested by removing developing embryos from maternal control, i.e., in vitro culture. Attempts by Sheridan and Neuffer (1982) to "rescue" lethal development mutants in maize using in vitro culture were not successful. Likewise, with human in vitro fertilization programs, P/Z ratios have not improved beyond the 20–25% level, suggesting that a genetically controlled limit has been reached (R. Urry, personal communication). Such limitations in cell culture success could be the result of as yet unidentified technical problems, but such a thesis is rarely falsifiable. The high levels of spontaneous abortion in humans (ca. 70%) is generally considered to be under genetic control (Biggers 1981). There is no doubt of this in the case of chromosomal mutations. In summary, we suggest that obvious aberrations among developing embryos are the result of lethal genes, as are various abnormalities occurring in other phases of the life cycle, e.g., the chlorophyll lethals in seedlings of grasses (Apirion and Zohary 1961). Such lethal genes are irreversible expressions of the zygotic genome.

Developmental selection. Embryo abortion in outcrossing flowers with multiovular ovaries could also be attributed to the outcome of competition among ovules with genetically diverse embryos, i.e., developmental selection (Buchholz 1922). Although no definitive data are available, developmental selection is strongly inferred from the wide range of sizes noted in the aborting ovules of many species. According to this relativistic model the least vigorous embryos would abort as the result of changing resource allocations among the competing embryos. Such a pattern could result from a positive feedback system based on the initial vigor of the particular embryos. There is likewise no reason why such a relativistic model could not work in combination with the absolutistic model of genetic load, i.e., some embryos must inevitably abort as the result of developmental lethals, whereas others may die as the result of competitive interaction. Embryos with differing numbers of deleterious or sublethal alleles might well die or survive, depending on threshold values in the ovary.

Abortion events due to genetic load might be distinguishable from those of developmental selection because of the gross morphological aberrations or cellular malfunctions that developmental lethals often display. Competitive embryonic death, however, might result from slow attrition without obvious morphological manifestations, but this need not always be the case. Developmental selection might explain the presence of smaller ovules with slowed growth rates noted in our material and also reported by other workers (Cooper et al. 1937); whatever the mechanism for competition induced embryo abortion, there was no obvious indication that it was caused through anatomical breakdown of the vascular system supplying the individual ovules in *E. angustifolium*. These purely mechanistic hypotheses, on parsimonius grounds, include no features implying active "female choice".

(1) *Maternal control (fixed abortion systems).* If maternal control is defined as a fixed characteristic determined by the maternal genome, then instances in which either the number and/or the position of maturing ovules remain constant are clear examples of maternal control. During an extensive survey of about 200 species of inbreeding and outcrossing plants, a number of such examples were identified from the Utah flora (Wiens 1984, and unpublished work).

In several species of inbreeders the number of maturing ovules was not only reduced to one by abortion, but the position of the single developed ovule was also fixed. Examples include: *Medicago lupulinus, Melilotus alba, M. officinalis,* and *Trifolium repens. Nemophila breviflora* also matures only a single ovule, but it is unknown if the position is fixed.

In several outcrossing species the number of developing ovules is also reduced to one by abortion, but the position of the surviving ovule is random in these examples. This situation was first studied in detail by Casper and Wiens (1981) in *Cryptantha,* but has been subsequently observed by us in a number of other genera: *Acer, Betula, Cornus, Dalea, Fraxinus, Geranium, Larrea, Pachistima, Prunus* (and all members of the Prunoideae), *Quercus, Symphoricarpos.* Other examples are given in Casper and Wiens (1981) and the condition no doubt occurs in many groups. Many cases are also known where specific ovule abortion is not fixed, but that ovules in certain positions on the placenta are statistically more prone to abortion (Horovitz et al. 1976).

Several critical points emerge from these observations: among consistent inbreeders the position of a maturing ovule can be fixed because the embryos are presumably genetically identical. Thus it is selectively irrelevant which ovule matures. Conversely, among outcrossing species the embryos are presumably genetically variable, and the expression of genetic load-developmental selection remains possible. In both cases selection for single-seeded fruits is related to dispersal and occurs mostly in species with dry, indehiscent fruits (Casper and Wiens 1981), although some species with this pattern of fixed abortion also have fleshy fruits (Wiens, unpublished data). In some fleshy-fruited species the abortion pattern is sometimes confined to individual carpels, which are then presumably the unit of interovular competition. Thus in *Symphoricarpus* or *Cornus* only one of several ovules mature in each of the two carpels, so the developed fruits actually have two seeds. The inbreeding species, *Erodium cicutarium,* has two ovules in each schizocarp, but only one develops. Embryological evidence from *Cryptantha* (Casper and Wiens 1981, Casper 1983) shows that in outcrossing species with a fixed rate of random abortion, the aborting ovules never progressed beyond the globular stage. Preliminary evidence from *Melilotus* (Calvin and Wiens unpublished data), an inbreeding species where both the number and position of aborting ovules are fixed, also indicates that fertilization occurs in all the ovules, but that the aborting embryos likewise do not develop beyond the globular stage. The mechanism of ovule abortion in such instances is clearly under fixed genetic control of the maternal plant, and appears to involve blockage of embryo development at an early stage.

Fixed abortion of specific fruits, and probably also of whole inflorescences no doubt occur, but we are unaware of examples.

(2) *Maternal control (fertilization).* The most obvious and well-known maternal control of fertilization are the incompatibility systems that discriminate among pollen grains of differing genetic constitution. A number of investigators, but particularly Mulcahy and his co-workers report that competition among pollen tubes improves seed "quality"

(Mulcahy 1978). Stephenson and Bertin (1983) further suggest that maternal influences might also influence which pollen tubes effect fertilization. From these observations the concept of a "genetic sieve" has arisen which would operate between pollination and fertilization. Although seeds are apparently improved through pollen tube competition among outcrossers, it does not insure that fertilization by vigorously growing pollen tubes produces high seed set. For example, in *E. angustifolium* hand-pollinated flowers regularly aborted around 50% ($\pm 20\%$) of the fertilized ovules. It must be remembered, however, that the female gametophyte is not subject to such prezygotic screening and thus might be more apt to contribute deleterious alleles to the zygote than the male. Without pollen tube competition, however, seed set would presumably be still lower (Lee 1984).

(3) *"Female choice" (seed and fruit production)*. Studies of "female choice" have not generally focused on seed development, although seed production should be influenced by "female choice" if sexual selection is applicable to plants (Janzen 1977; Charnov 1979; Willson 1979). Stephenson and Bertin (1983) in their excellent review mention only the study of *Cryptantha* by Casper and Wiens (1981) discussed earlier.

The primary focus has been on fruit set, where a sigificant literature is developing. No attempt is made to review these studies comprehensively (see Stephenson 1981; Stephenson and Bertin 1983; for earlier reviews, and the more recent work of Sutherland 1986; Stephenson and Winsor 1986; Marshall and Ellstrand 1986; and the references cited therein).

Maturing ovules are known to produce growth regulators which, in turn, stimulate fruit development. Likewise, young fruits with a greater number of developing ovules are more apt to mature, presumably because they produce more growth regulators and consequently grow faster, than ovaries with fewer developing ovules (Stephenson 1981). This positive feedback system presents a straightforward, mechanistic model that can generally explain fruit abortion; it is also testable. Those fruits with a greater number of developing ovules should be expected to outcompete those with fewer maturing ovules, particularly if resources are limiting. We submit that it is the number and vigor of the developing ovules and their embryos that determine fruit survivorship, i.e., causal events within the ovary control fruit maturation.

One of the best studies supporting "female choice" is by Stephenson and Winsor (1986). They showed that in *Lotus corniculatus*, which normally aborts 50% of its fruits and is presumably resource limited, seeds produced from naturally matured fruit were superior in various aspects to those obtained from shoots in which 50% of the fertilized flowers were hand-thinned.

Stephenson and Winsor posit pollen tube competition as the mediating mechanism to account for the correlation observed between average seed set/fruit and the "quality" of the progeny derived from those seeds. They argue that in their system, *Lotus corniculatus*, pollination is limiting for seed set/fruit because the average seed set/fruit in naturally-thinned shoots (ca. 50%) is significantly higher than in control shoots systematically hand-thinned to the same degree. However, if one assumes a distribution for the number of developing ovules (few-many) in the ovaries of

L. corniculatus, those fruits from the naturally-matured group would be positively skewed toward the high side of the distribution, i.e., the overall sample was composed of a larger number of fruits that contained a greater number of matured seeds. This result is explicable by the growth regulator model. Those young fruits with a greater number of developing ovules produced more hormones resulting in faster growth rates and/or greater sink capacity for nutrients. This ultimately allowed the fruits with more developing ovules to outcompete those fruits with fewer developing ovules when nutrients are limiting. In the experimentally hand-thinned group, this nutrient limitation is substantially relaxed so that most of the remaining ovaries, irrespective of their developing ovule content, mature. Thus, the fruits from the hand-thinned group represent a random sample of the initial ovary population, resulting in a lower mean seed set per fruit than in the naturally-thinned group apart from Stephenson and Winsor, others who support this position, are persuaded to hypothesize the involvement of "female choice" because by the criteria of vegetative growth and reproductive vigor, the progeny derived from the naturally-thinned group are significantly superior to those from the artificially hand-thinned group.

We suggest that the orthodox growth regulator dependent fruit persistence model in conjunction with a corollary of our genetic load-developmental selection hypothesis could just as well account for Stephenson and Winsor's results.

Given the habits of pollen vectors it is very likely that the paternity of each ovary represents a restricted sample of the pollen parents available to any given plant. Thus, one would expect a significant degree of variation among the ovaries on a given plant with respect to the particular paternity of each. Therefore, different fruits on the same plant in effect provide a progeny test of the various pollen parents available to that plant. That is, much of the variation in seed set/fruit among the fruits on one plant should be contributed by differences among the genotypes of the pollen parents pollinating that plant (Bertin 1982, Marshall and Ellstrand 1986). Such differences should influence the vigor and viability of the embryos derived from them.

We insist, however, that no active "female choice" is necessary to effect the progeny screening proposed here. This effect is simply the result of a passive mechanistic corollary of interembryonic competition operating within each ovary of a given plant, where the ovaries are heterogeneous with respect to pollen parentage. In this situation more seed should mature in ovaries with superior paternity. This, coupled with growth regulator control of subsequent fruit persistence, would generate a correlation between seed set/fruit and progeny vigor. Indeed, the issue of "female choice" aside, it now becomes critically important to make an assessment of the relative contribution of pollen tube competition and interembryo developmental selection (encompassing both failure of embryos because of their genetically based lethality, and embryo abortion mediated by interembryonic competition among the remaining potentially viable embryos). This could be approached by testing the effects of pollen competition by hand-pollination, while also controlling parental source on both seed set and the growth and reproductive characteristics of the progeny derived from them in Stephenson and Winsor's system.

Where maternal controls occur, such as in cases of fixed position ovule abortion, or in incompatibility systems, they

are characterized by nonrandomness in terms of where the aberrant embryos abort, and probably also the stage of development at which they fail. Conversely, the outcome we attribute to the genetic constitution of the zygotic genome results in randomness of embryo abortion in both space and time, as suggested by Stephenson and Bertin (1983).

If "female choice" generally determines seed and fruit survivorship, by what mechanisms might it operate? A "female choice" hypothesis (in its extreme form) assumes that a plant is capable of (1) evaluating the abiotic and biotic environments into which a seed is apt to be dispersed, (2) determining those genotypes that best fit the requirements, and (3) specifically aborting those embryos which do not meet the criteria. How might a plant acquire, transfer, evaluate, and implement such information? And what are the implications? Considerable rethinking of selection theory is certainly necessary. As H.G. Baker (personal communication) points out, if "female choice" produces "quality" seeds, it is unlikely that the vigor of the embryo alone will insure that the resulting plants will be better adapted to the environment that it enters. This involves the seedling and mature plant encountering climatic, edaphic, and biotic factors that have no apparent relevance to conditions in the ovule.

A final question, what *is* the definition of the term "quality" in its evolutionary context?

Conclusions

The embryological data in particular suggest to us that genetic load and developmental selection provide the best explanations for reduced fruit and seed set reduction in *E. angustifolium* and outcrossing species generally. While resource availability and pollination may be important in some situations, we found little evidence that they played an important role in limiting reproductive success in unstressed natural populations. We would expect resource limitations to affect seed or fruit set in cases of more extreme, and especially unpredictable instances of environmental perturbations, e.g., heavy predator, or epidemic pathogen activity, sudden drought, or loss of photosynthate. But this is no reason to suggest that it will be the "best" progeny that will be saved under such circumstances. We have evidence of S/O ratios reduced up to 70% due to environmental stress in inbreeding species such as *E. ciliatum* and *Capsella bursa-pastoris*, but the stress effect has not been quantified. Conversely, enhancement of both the Fr/Fl and P/Z ratios might also occur during periods of optimal reproductive age or resource availability when thresholds for the expression of sublethals are raised and the ability to tolerate deleterious alleles are improved (Janzen 1977, Willson and Burley 1983). These, however, are likely only minor variations within the broad patterns of PERS dictated by genetic load and/or developmental selection. The high variation (S.D.$\pm$ca. 20) in both the Fr/Fl and P/Z ratios among species, populations, and individuals warrants further study. If these variances were due to environmental factors, the co-occurring and congeneric inbreeding species should exhibit similar effects, which they do not.

Various examples of reduced seed set can be considered a consequence of the combination of genetic load-developmental selection associated with normally outcrossing plants. Inbreeding depression and "delayed self-incompatibility", i.e., early embryo death in selfed plants that normally outcross, are such examples (Seavey and Bawa 1986). It may, however, be difficult to distinguish genetic load from self-incompatible alleles during inbreeding. "Outcrossing depression" (Waser and Price 1983) is also a probable effect of genetic load, especially in the case of intrapopulational crosses among typical inbreeders (Vickery 1978). There seems to be no reason why these phenomena should not be included under a single unified concept of genetic load-developmental selection.

The surprisingly low PERS in outcrossing species of *Epilobium* and outcrossing flowering plants generally, must be due to genetic load-developmental selection of an intensity comparable to that reported by Sorensen (1969) for Douglas fir. Similarly, low P/Z ratios in humans (Biggers 1981) and rabbits (Brambell 1948) suggest that PERS is a generally applicable, simple, and direct expression of genetic load and/or developmental selection, and that its magnitude is considerably greater than generally assumed.

PERS is essentially a measure of selective forces acting on the embryo phenotype during development. Our data show that among outcrossing genetically heterozygous organisms a greater proportion of potential progeny are lost during the preemergent phase of the life cycle than the postemergent phase, as Haldane (1957) suggested might be the case nearly three decades ago. Conversely, among obligate inbreeding, genetically homozygous organisms, selection is primarily postemergent.

References

Apirion D, Zohary D (1961) Chlorophyll lethals in natural populations of orchard grass (*Dactylis glomerata* L.): a case of balanced polymorphism in plants. Genetics 46:393–399

Bertin RI (1982) Paternity and fruit production in trumpet creeper (*Campsis radicans*). Am Nat 119:694–709

Biggers JD (1981) In vitro fertilization and embryo transfer in human beings. New Engl J Med 304:336–342

Bradbury D (1929) A comparative study of the developing and aborting fruits of *Prunus cerasus*. Amer J Bot 16:525–542

Brambell FWR (1948) Prenatal mortality in mammals. Biol Rev 23:370–407

Browder LW (1984) Developmental Biology. Saunders, New York

Buchholz JT (1922) Developmental selection in vascular plants. Bot Gaz 73:249–286

Casper BB (1983) The efficiency of pollen transfer and rates of embryo initiation in *Cryptantha* (Boraginaceae). Oecologia (Berl) 59:262–268

Casper BB, Wiens D (1981) Fixed rates of random ovule abortion in *Cryptantha flava* (Boraginaceae) and its possible relation to seed dispersal. Ecology 62:866–869

Charnov E (1979) Simultaneous hermaphroditism and sexual selection. Proc Nat Acad Sci USA 76:2480–2484

Cooper DC, Brink RA, Albrecht HR (1937) Embryo mortality in relation to seed formation in alfalfa (*Medicago sativa*). Amer J Bot 24:203–213

Essau K (1977) Anatomy of Seed Plants. John Wiley & Sons, New York

Flint EP (1980) Ecology and distribution of diploid and tetraploid *Epilobium angustifolium* (fireweed) in the Beartooth Mountains of Wyoming and Montana. Ph.D. Thesis, Duke University

Hadorn E (1961) Developmental genetics and lethal factors. Translated by V. Mittwoch, Methuen and Co Ltd., London

Haldane JBS (1957) The cost of natural selection. J Genetics 55:511–524

Herr JM, Jr (1974) A clearing-squash technique for the study of ovule and megagametophyte development in Angiosperms. In: Radford AE, Dickinson WC, Massey JR, Bell CR (eds) Vascular Plant Systematics. Harper & Row, New York

Horovitz A, Meiri L, Beilles A (1976) Effects of ovule positions in fabaceous flowers on seed set and outcrossing rates. Bot Gaz 137:250–254

Janzen DH (1977) A note on optimal mate selection by plants. Amer Nat 111:365–371

Johri BM (1963) Female gametophyte. In: Maheshwari P (ed) Recent Advances in the Embryology of Angiosperms, pp 59–103. International Soc of Plant Morph, Delhi

Klekowski EJ, Jr, Kazarinova-Fukshansky N, Mohr H (1985) Shoot apical meristems and mutations: stratified meristems and angiosperm evolution. Amer J Bot 72:1788–1800

Lee TD (1984) Patterns of fruit maturation: a gametophyte competition hypothesis. Amer Nat 123:427–432

Marsden MPF, Meinke DW (1985) Abnormal development of the suspensor in an embryo-lethal mutant of *Arabidopsis thaliana*. Amer J Bot 72:1801–1812

Marshall DL, Ellstrand NC (1986) Sexual selection in *Raphanus sativa*: experimental data on non random fertilization, maternal choice, and consequences of multiple paternity. Amer Nat 127:446–461

Meinke DW, Sussex IM (1979) Isolation and characterization of six embryo-lethal mutants of *Arabidopsis thaliana*. Dev Biol 72:50–61

Meinke DW (1985) Embryo-lethal mutants of *Arabidopsis thaliana* with a wide range of lethal phases. Theor Appl Genet 69:543–552

Mosquin T (1966) A new taxonomy for *Epilobium angustifolium* (Onagraceae). Brittonia: 167–188

Mulcahy DL (1978) Further evidence that gametophytic selection modifies the genetic quality of the sporophyte. Soc Bot Fr Actualites Bot n1-2:57–60 (references therein)

Muller HJ (1950) Our load of mutations. Amer J Human Genetics 2:111–176

Muller HJ (1964) The relation of recombination to mutational advance. Mutat Res 1:2–9

Sang JH (1984) Genetics and Development. Longman, New York

Schoen DJ (1981) The evolution of self-pollination in *Gilia achilleifolia* (Polemoniaceae). Ph. D. thesis, Univ Cal, Berkeley

Schoen DJ (1982) Genetic variation and the breeding system of *Gilia achilleifolia*. Evolution 36:361–370

Seavey SR, Bawa KS (1986) Delayed self-incompatibility in Angiosperms. Bot Rev 52:195–219

Sheridan WF, Neuffer MG (1982) Maize development mutants, embryos unable to form leaf primordia. J Heredity 73:318–329

Sorensen F (1969) Embryonic load in coastal douglas-fir, *Pseudotsuga menziesii* var. *menziesii*. Amer Nat 119:694–709

Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. Ann Rev Ecol Syst 12:253–279

Stephenson AG, Bertin RI (1983) Male competition, female choice, and sexual selection in plants. L.A. Real (ed) Pollination Biology, Academic Press, NY

Stephenson AG, Winsor JA (1986) *Lotus corniculatus* regulates offspring quality through selective fruit abortion. Evolution 40:453–458

Sutherland S (1986) Patterns of fruit-set: what controls fruit-flower ratios in plants. Evolution 40:117–128

Vickery RK (1978) Case studies in the evolution of species complexes in *Mimulus*. In: Hecht MK, Steere WC, Wallace B (eds) Evolutionary Biology 11:405–507. Plenum, New York

Waser NM, Price MV (1983) Optimal and actual outcrossing in plants, and the nature of the plant-pollinator interaction. In: Jones CE, Little RJ (eds) Handbook of Experimental Pollination Biology, pp 341–359. Van Nostrand Rheinold, New York

Wiens D (1984) Ovule survivorship, brood size, life history, breeding systems and reproductive success in plants. Oecologia (Berl) 64:47–53

Willson MF (1979) Sexual selection in plants. Amer Nat 113:777–790

Willson MF, Burley N (1983) Mate choice in plants. Princeton Univ Press, Princeton