

SUPPLEMENTAL MATERIALS

Here, we provide details on the calculations of individual components of reproductive isolation within each study system, as well as pertinent references. In reciprocal transplant experiments where multiple measures of fitness (e.g. size, mass, growth, survival, etc.) were assayed in the same experiment but a composite measure of fitness was not presented, we used survival as the most direct means of calculating immigrant inviability. For studies providing composite measures of fitness, we used total fitness for our calculations.

For *Acyrtosiphon pisum*, host preference was estimated as the mean from two experiments: one examining the distribution of diagnostic allozyme loci between fields of alfalfa and clover and one examining the tendency for aphids from each host race to settle on alfalfa versus clover in laboratory choice experiments (Via 1999). These two experiments gave comparable estimates of host preference (0.889 and 0.965 respectively). Their mean was used in all calculations in the current study. The fitness (fecundity) of immigrants versus residents was estimated in a reciprocal transplant experiment where winged forms (i.e. the natural dispersers) of a set of aphid genotypes from each host were experimentally ‘migrated’ to one of the two crops. Hybrid ecological fitness was estimated as the fecundity of F1 hybrids relative to each parental form in its native habitat (Via et al. 2000).

For *Agelenopsis aperta*, immigrant inviability was estimated using survival data from Riechert and Hall 2000. Sexual isolation was estimated using the laboratory mating trials reported in Riechert et al. 2001. Genetic Incompatibility was estimated using the data on spiderling mass and survival to sexual maturity for parental and F1 hybrid crosses reported in Riechert et al. 2001 (averaged across these two measures of fitness). Notably, differences among genetic classes in these latter two forms of reproductive isolation were not statistically significant.

For *Artemisia tridentata*, (Wang et al. 1997), immigrant inviability was estimated using the composite fitness of native versus foreign plants in each parental environment (basin and mountain). Natural selection against hybrids was estimated by comparing the composite fitness of hybrids versus each parental form (averaged over both parents) within the hybrid environment, where hybrids were much more fit, resulting in negative isolation values.

For *Bombina* spp., clear evidence for divergent habitat preferences has been obtained using allele-frequency data, rather than by habitat-preference experiments. We used the estimated difference in allele frequencies at habitat preference loci between puddles and ponds as an indirect estimate of the degree of habitat isolation between puddle-adapted *B. variegata* and pond-adapted *B. bombina* (see MacCallum et al. 1998, p. 237 for details). Immigrant inviability was also difficult to estimate because a reciprocal transplant experiment suggested that immigrants were selected against in one environment (puddles) but favored in another (ponds) when predators were excluded (MacCallum et al. 1995). However, predation trials indicated that immigrants would be selected against in ponds when predators were present ((Kruuk and Gilchrist 1997). Furthermore, the reciprocal transplant experiment was flawed due to mixed species egg batches. We adopted the conservative strategy of assigning immigrants and residents equal fitness in ponds and dividing the estimate of immigrant inviability from puddles in half to yield a rough estimate averaged across the two environments. Intrinsic hybrid inviability was assessed by comparing the viability of egg batches from the center of a hybrid zone to that of egg batches from parental types, with viability estimated using total mortality, the product of embryonic and larval mortality for each batch (as in Kruuk et al. 1999a).

For *Eurosta solidaginis*, host preference was estimated as the mean from three experiments (0.974, 0.969 and 0.942 for no-choice host preference, choice host preference, and mating site host preference experiments respectively (Craig et al. 1993)). The survival of immigrants versus residents on each host was estimated from the proportion of eggs oviposited on each host that yielded adults. Sexual isolation was taken as the mean from two experiments where mating preferences were measured in the absence of hosts, including one no-choice scenario and one multiple-choice scenario (Craig et al. 1993). A lack of intrinsic inviability was inferred from consistently high hybrid survival on some plant genotypes of both host-plant species (Craig et al. 1997; Itami et al. 1997; Craig et al. 2001). Ecological hybrid inviability was inferred by comparing emergence success (proportion of eggs oviposited on each host that yielded adults) of hybrids to that of each parental form on its native hosts (with hybrid fitness averaged across the two hosts, Craig et al. 1997).

For *Galerucella nymphaeae*, all data were taken from Pappers et al. 2002a. (Although Pappers et al. 2002b report similar data and nearly identical results, the former study was much larger and controlled in some cases for environmental and maternal effects.) In all instances, results were averaged across the four treatments (two Nymphaeaceae hosts and two Polygonaceae hosts). Habitat preference was calculated from the results of an oviposition preference experiment, and immigrant inviability was calculated from the survival of insects on hosts from their native versus foreign host family. Sexual isolation and genetic hybrid inviability were considered to be absent because all types of crosses (within and between host family) interbred equally and hybrid offspring outperformed parental forms when fitnesses were averaged across all treatments.

For *Galerucella* spp., we used data from Nokkala and Nokkala (1998) to estimate components of reproductive isolation between *G. nymphaeae* and *G. sagittariae*, in all cases averaging the results from the two forms of *G. sagittariae* that were examined. Habitat isolation was estimated from habitat choice tests where insects were scored for the host on which they were sitting and on which they oviposited, with results averaged across sitting and oviposition assays. Percent larval survival of each parental form and of hybrids on each host provided estimates of immigrant inviability and ecological hybrid inviability. All hybrids were fertile, indicative of a lack of genetic hybrid inviability.

For *Gasterosteus aculeatus*, estimates of immigrant versus resident fitness were averaged across three studies. Schluter (1995) and Rundle (2002) provide estimates of growth rates of benthic and limnetic forms in each habitat, whereas Vamosi (2002) examined survival under predation. These studies gave comparable estimates (0.418, 0.393, 0.280 respectively) and were chosen because the fitness of both limnetic and benthic fish were measured in both habitats in natural populations. Studies using laboratory feeding trials, semi-natural pond experiments, or that performed experiments in only one habitat were excluded but yielded similar conclusions (Schluter 1993, 1994; Hatfield and Schluter 1999; Vamosi et al. 2000; Vamosi and Schluter 2002). Sexual isolation was estimated by comparing the probability of spawning between males and females from the same lake and same ecotype to the probability of spawning between different ecotypes from the same lake (data from Rundle et al. 2000, which include data from Nagel and Schluter 1998). Hatfield and Schluter (1999) found no evidence for genetic hybrid inviability, and assessed growth rate of F1 hybrids versus each parental form in its native habitat. Analogous data from Schluter 1995 is for F10 hybrids and thus was excluded, but trends were similar. With respect to sexual selection against hybrids, a recent field experiment found reduced mating success of F1 hybrid males relative to limnetic males (Vamosi and Schluter 1999),

indicating that the lack of a hybrid mating disadvantage found in laboratory mating trials by Hatfield and Schluter (1996) does not apply in the wild; the latter study was thus excluded.

For *Gilia capitata*, selection against immigrants was estimated using data on the proportion of immigrant versus resident plants to flower, averaged across the multiple years of the experiment (Nagy and Rice 1997). Whether pollen competition occurs between subspecies is unclear, but hybridization rates are only 20% of that expected based on the pollen mixture applied in a greenhouse experiment, indicative of a fertility barrier between inland subspecies male function and coastal subspecies female function (see Nagy 1997a, p. 711, for discussion). Genetic hybrid inviability was estimated from mean number of seeds per fruit for parental versus F1 hybrid crosses (Nagy 1997a). Ecological hybrid inviability has been documented (Nagy 1997b), but not in a manner that allows a standardized index of hybrid versus parental fitness to be calculated.

In *Heliconius erato* postman and rayed mimetic races, Mallet (1989) noted a lack of host preferences. Ecological selection against immigrants was quantified using estimates of the longevity of immigrants and residents. A lack of sexual isolation was inferred from no evidence for assortative mating in crosses (Mallet 1989), while population genetic analyses indicated random mating and a lack of hybrid inviability in the field (Mallet et al. 1990). Breeding experiments indicated full fertility and viability of hybrids, backcrosses, F2, and future hybrid generations (Mallet 1989). Ecological hybrid inviability was estimated using the probability of establishment of hybrids versus residents (i.e. the local race) at a single site (Mallet and Barton 1989).

For *Ipomopsis spp.*, immigrant inviability was estimated from the percentage of plants surviving to flower or to five years of age for immigrant and resident species at four parental sites (Campbell and Waser 2001). A lack of genetic hybrid inviability was evidenced by the equal means of seeds per flower produced by hybrid versus pure species crosses (Campbell and Waser 2001; Campbell et al. 2003). Floral isolation is also likely in this system (see Campbell 2003 for review), but published data did not allow us to easily construct an appropriate index. Ecological hybrid inviability was estimated by comparing the percentage of plants surviving to flower or to five years of age for hybrid versus parental species in a hybrid environment in a reciprocal transplant experiment (Campbell and Waser 2001). Sexual selection against hybrids was estimated by comparing the number of seeds per fruit produced by crosses where the pollen was purely from hybrids versus mixed pollen of hybrids and *I. aggregata* (Campbell et al. 2003).

For *Iris spp. 1*, overlap in flowering times indicated a lack of temporal isolation (Young 1996); immigrant inviability was estimated from survivorship of reciprocally-transplanted individuals, a lack of floral isolation was inferred from pollinator observations, and genetic hybrid inviability was estimated from the seed set of parental and hybrid crosses from controlled pollination experiments (in which hybrids outperformed parentals but not significantly so, Young 1996).

For *Iris spp. 2*, immigrant inviability was estimated using the survival of immigrant versus resident species in each parental habitat (averaged across the two parental habitats) and ecological hybrid inviability was estimated by comparing the survival of F1 hybrids to each parental form in each of the two hybrid sites (averaged across sites and parental forms, Emms and Arnold 1997).

In *Littorina saxatilis* ecotypes, direct tests of habitat preference were unavailable, but migratory differences were quantified between ecotypes. The tendency for smooth unbanded morphs from the lower shore to move towards the sea when transplanted to the upper shore and

the tendency for ridged and banded morphs from the upper shore to move towards land when transplanted to the lower shore were thus used as a measures of habitat isolation (Erlandsson et al. 1998). This tendency was estimated using the mean angular dispersion for each morph, averaged across treatments, when transplanted to the foreign shore (Erlandsson et al. 1998 for details). Immigrant inviability was estimated using cross-product estimates of the viability of each pure morph at upper and lower shore levels, using only morphs originating from these two shore levels. Viability estimates pooled among sites were used and were averaged across four comparisons (small individuals on upper and lower shores, large individuals on upper and lower shores). Estimates of sexual isolation came from two sources (both contained in Rolan-Alvarez et al. 1999, which includes data from Johannesson et al. 1995). First, total assortative mating in natural populations was found to be 0.71 in summer and 0.75 in the fall, but arises from a combination of microspatial differences and divergent mating preferences among morphs. Computer simulations showed that about 40% and 31% of the total isolation comes from microhabitat differences for summer and fall samples, respectively, requiring individual components of 0.52 and 0.64 for sexual isolation to yield the observed levels of total assortative mating (mean individual component = 0.58). A laboratory estimate of sexual isolation was based on small sample sizes, but was comparable (0.46). We used the mean from the field and lab estimates of sexual isolation. There was no evidence for genetic hybrid inviability (Rolan-Alvarez et al. 1997; Cruz et al. 1998; Johannesson et al. 2000). We include estimates of ecological hybrid inviability, but note that results were not statistically significant because hybrids exhibited high survival in the midshore region where they are found (midshore results were only used for this component of isolation; Rolan-Alvarez et al. 1997). Finally, although sexual selection against hybrids was not detected in a recent study (Cruz et al. 2001), there was some evidence that extreme forms within hybrids (i.e. those resembling each parental morph) did have higher mating success.

For *Mimulus* spp., all data are from Ramsey et al. (2003), who estimated multiple components of reproductive isolation between species of monkeyflowers. We used their measure of ecogeographic isolation as an estimate of immigrant inviability (see Ramsey et al. 2003 for details) but congruent results were obtained by Hiesey et al. 1971. We also used their estimates of reproductive isolation caused by pollinator fidelity (i.e. floral isolation), pollen competition, and total postzygotic isolation. We provide this estimate of total postzygotic isolation in the genetic hybrid inviability category but note that it is a composite measure of postmating isolation that includes measures of fitness that might be considered natural or sexual selection against hybrids, e.g., pollen viability of hybrids (see Ramsey et al. 2003 for details). In all cases, we used the average for the two species.

For *Mitoura* spp., data are from Forister 2004, who examined oviposition preference and larval performance of three host races (Cedar, Juniper and Cypress races) on four different hosts (one Cedar species, one Juniper species and two Cypress species). Oviposition preference provided a measure of habitat isolation and was estimated as percent ovipositing on their native host, averaged across the different populations within a host race category and then averaged across host races. Larval survival provided a measure of immigrant inviability and for simplicity we have averaged the results such that immigrant inviability is estimated as the mean relative survival of all the other host races on the native host of a race, averaged across the three host races. The different host races can freely interbreed in the lab and produce fertile offspring, indicative of a lack of genetic incompatibility (Forister unpublished).

In *Neochlamisus bebbiannae*, habitat isolation was estimated as the average population level host preference from six experiments, all of which gave comparable results (see Funk 1998 for details; means across populations from each experiment: percent eggs laid on non-native host = 7.08; percent of time spent on non-native host = 12.86; larval feeding on non-native host under no-choice conditions = 8.54; larval feeding on non-native host under choice conditions = 1.20; adult feeding on non-native host under no-choice conditions = 1.88; adult feeding on non-native host under choice conditions = 0.50). Immigrant inviability was estimated by comparing the survival of larvae transplanted to their non-native host to the survival of larvae reared on their native host. Sexual isolation was inferred from the isolation indices reported in Funk 1998 (Levene's isolation index, transformed to a scale of zero to one).

For *Polemonium viscosum*, immigrant inviability was estimated using the survival of immigrant versus resident plant ecotypes in each of two environments in a reciprocal transplant experiment (Galen et al. 1991). Floral isolation was estimated indirectly from information on (1) the relative frequency of different floral scent morphs at low versus high elevation sites, and (2) the seed set of naturally pollinated plants of each morph at each elevation (i.e. 'floral sexual fitness'). One-third of the individuals at low sites were the sweet morph and two-thirds were the skunk morph, whereas at the high elevation site two-thirds of the individuals were the sweet morph (Galen and Kevan 1980). At the low site, the sweet and skunk morphs produced 1.37 versus 2.67 seeds per flower, respectively, and at the high site 2.73 versus 1.84 seeds per flower, respectively. Thus, immigrants to the low site were assigned a floral sexual fitness of: one-third \times 2.67 + two-thirds \times 1.37, whereas residents of that site were assigned a floral sexual fitness of: one third \times 1.27 + two-thirds \times 2.67. Migrants to the high site were assigned a floral sexual fitness of: one-third \times 2.73 + two-thirds \times 1.84, whereas residents of that site were assigned a floral sexual fitness of: one third \times 1.84 + two-thirds \times 2.73. Although reduced seed set of the less-frequent morph at each site might reflect pollen competition or postzygotic isolation, floral isolation seems more likely based upon pollination and weeding experiments (see Galen 1985 for details).

For *Rhagoletis spp.*, habitat isolation was estimated from percent oviposition on apple versus blueberry in no-choice trials, immigrant inviability from percent larval survival to adulthood of each species on each host, and ecological hybrid inviability from percent larval survival to adulthood for hybrids versus parental forms in their native environment (all data from Bierbaum and Bush 1990).

Methodology for *Timema cristinae* walking-stick insects is given in detail in Nosil et al. 2002, 2003 (sexual isolation) and Nosil 2004 (immigrant inviability). For sexual isolation, mating frequencies for parapatric and allopatric populations were pooled to estimate homo- and heterotypic mating frequencies. The strength of immigrant inviability was estimated using the mean survival of between-host immigrants versus residents averaged between allopatric and parapatric pairs of populations. Host preference data are from Nosil (unpublished), who has conducted choice experiments in the lab. In all cases, only populations using alternate host-plant species were used in the calculations.