A Selective Advantage to Immigrant Genes in a *Daphnia* Metapopulation

Dieter Ebert,1,2,3* Christoph Haag,1,2,3* Mark Kirkpatrick,4 Myriam Riek,1,2 Jürgen W. Hottinger,1,2,3 V. Ilmari Pajunen2,5

Immigrants to habitats occupied by conspecific organisms are usually expected to be competitively inferior, because residents may be locally adapted. If residents are inbred, however, mating between immigrants and residents results in offspring that may enjoy a fitness advantage from hybrid vigor. We demonstrate this effect experimentally in a natural *Daphnia* metapopulation in which genetic bottlenecks and local inbreeding are common. We estimate that in this metapopulation, hybrid vigor amplifies the rate of gene flow several times more than would be predicted from the nominal migration rate. This can affect the persistence of local populations and the entire metapopulation.

Gene flow between populations can be both a creative and a constraining force in evolution (1–3). The introduction of new genetic material into a population increases local genetic diversity and helps the spread of favorable alleles across metapopulations. On the other hand, it reduces genetic variation between populations and hinders local adaptation. In inbred populations, the consequences of migration may be particularly important: If the hybrid offspring of immigrants and residents are competitively superior, their hybrid vigor will amplify the gene flow caused by migration (4–6). Furthermore, the demographic consequences of increased vigor could prevent the decline and even the extinction of populations (7–12). The magnitude of hybrid vigor is, however, controversial. Highly inbred populations may have low genetic loads because inbreeding exposes recessive deleterious alleles to purging by natural selection (13–17).

In subdivided populations with local extinctions and colonizations, genetic bottlenecks can be frequent, leading to increased homozygosity (15, 16, 18). If homozygosity results in a fitness reduction (inbreeding de-
In 1998, a population genetic survey of 96 *D. magna* populations with known age indicated that local populations are often found by a single clone, followed by a population increase due to clonal expansion (24). Such genetic bottlenecks lead to substantial inbreeding during sexual reproduction, which is most extreme when a single founder clone produces resting eggs by mating with clonal brothers and sisters (known as selfing) (25). The frequent occurrence of genetic bottlenecks makes this metapopulation an ideal site to study the impact of local inbreeding on the success of immigrants.

To test whether hybrid vigor occurs in this metapopulation, we introduced immigrant genotypes into 22 *D. magna* populations and monitored the fate of their outbred offspring over one summer season. We chose 22 rock pool populations on nine islands in July 1999. These rock pools were known to be continuously inhabited by *D. magna* for at least 2 years before the beginning of the study. Of the five genotypic loci known to be polymorphic in these metapopulations, the 22 studied populations were polymorphic at none (12 populations), one (six populations), or two loci (four populations), which is typical of the metapopulation. In July 1999, we removed all *D. magna* from these populations and kept 200 to 500 females from each population with minimal selection in the laboratory. To remove resting eggs that may have been produced earlier in that season, we removed the water and most of the soft sediments from the pools. Comparable disturbances occur naturally in this metapopulation, where storms on the Baltic Sea frequently wash out parts of or even entire pools (19, 21). We also collected 22 clones from different pools within the study area and propagated them in the laboratory. These we designated as our experimental immigrant clones.

Two weeks later, after rain had refilled the pools, we brought 200 individuals of the populations back into their original pools and added 200 individuals of one immigrant clone into each pool (26). In each experimental pool, the immigrant clone differed from the local clones at least one allozyme marker locus. To avoid bias due to potential fitness effects of the genetic markers, we used multiple alleles at four loci. The genetic markers allowed us to distinguish between hybrids, offspring of local residents, and selfed offspring of the immigrant clone after the populations had undergone one round of sexual recombination, i.e., in spring of the following year.

Rock pools were left undisturbed until we took the first samples in May 2000. From these samples, we genotyped 66 to 122 animals and also founded laboratory populations using 200 to 300 females each. This was done to duplicate the natural rock pool experiment under controlled laboratory conditions so that we could decouple the effect of inbreeding from uncontrolled environmental effects, such as the local pool environment, predators, and further immigrants from the same or other *Daphnia* species (27). Second and third samples were taken from all populations about 60 and 100 days after the first samples had been collected.

Our hypothesis was that the outbred offspring would increase in frequency as the result of hybrid vigor. Figure 1 shows that this was the case in all rock pool populations where we recovered hybrids in May 2000 (all but pool 15). It was also the case in 17 of 18 laboratory populations (Fig. 1) (28). The changes in genotype frequencies in the field and the laboratory were highly correlated with each other (*r* = 0.71 to 0.93 for the three offspring types, *P* < 0.002, *n* = 16), and the increase of outbred genotypes did not differ significantly among them (paired *t* test; *t* = 0.52, *P* = 0.61, *n* = 16). Hybrids also increased significantly in the time period between the second and the third sample (*P* < 0.01 in the field and the laboratory) at a time when genotypes with very low fitness (e.g., due to castrating homozygous recessive mutations) had already been purged. Therefore, the observed hybrid vigor is at least partially due to deleterious effects of alleles with weak to intermediate effect (6, 29). As selection against such alleles is weak, they can accumulate to high frequencies. High genetic
loads have been reported for Daphnia (30–32).

We believe that hybrid vigor best explains our results, as a number of alternative hypotheses can be excluded. First, because we used a different immigrant clone for each population, it is unlikely that all of them carried superior alleles (33). Second, although rare genotypes could have had an advantage by, for example, exploiting unoccupied niches, we rejected this hypothesis because hybrids increased in frequency irrespective of their initial frequencies in May 2000 (Fig. 1). Third, although residents may suffer from locally adapted predators or parasites (34, 35), predators were excluded from the laboratory, and the increase of hybrids did not differ significantly between populations infected with a parasite or not (36). Fourth, mating success cannot explain our results (37–39) as hybrid success was measured during the asexual phase of the Daphnia life cycle.

One key effect of hybrid vigor is an increase in effective gene flow (4). We estimate that 2 years after a natural immigration event (where immigrants represent a much smaller fraction of the population than in our experiments), the effective rate of gene flow is about 35 times larger than would be predicted by the number of immigrants alone, and it will even increase further in the following years (40). The amount by which gene flow is amplified depends on the causes and magnitude of hybrid vigor. It may be much smaller in genetically more diverse Daphnia populations but may be high in other systems characterized by frequent extinction and recolonizations.

Amplified gene flow caused by hybrid vigor may account for lower observed levels of population differentiation than predicted by models based on neutral effects (4, 6, 41–43) and may influence the evolution of dispersal (44). An important effect of hybrid vigor is the “genetic rescue” of populations from extinction, because it may influence extinction and colonization dynamics of the whole metapopulation (5). Although we did not show a link between population persistence and inbreeding depression, such a relation has been shown before (9, 11, 12) and is likely to apply to Daphnia metapopulations as well. In this light, gene flow is an essential component for the persistence of metapopulations. Thus, our study gives clear empirical support for the need to maintain gene flow in the management and conservation of subdivided populations.

Reference and Notes
and that the fitnesses of later backcrosses converge geometrically on 1 at a rate of 1/2 per sexual generation (year). Using parameter values suggested for *Daphnia* (46) \( s = 0.14 \) and \( h = 0.3 \) and setting \( i_m = i_r = 55 \) to give an \( F_1 \) fitness similar to what we observed, we obtain \( m^*(2) = 35.7 \), \( m^*(3) = 234m \), and \( m^*(n) = 6703m \). Lower values of \( m^* \), but still substantially greater than 1, can be obtained when \( i_m \) and \( i_r \) have more modest values. Thus, hybrid vigor can make gene flow many times stronger than would be predicted from the nominal migration rate under these conditions.

47. We thank T. Kawecki, M. Ackermann, D. Meyer, and S. Zweizig for critical comments to earlier version of the manuscript. This work was supported by the Swiss Science Foundation and NSF grant DEB-9973221.