

# Rapid spread of immigrant genomes into inbred populations

Ilik J. Saccheri<sup>1,2\*</sup> and Paul M. Brakefield<sup>1</sup>

<sup>1</sup>*Institute of Evolutionary and Ecological Sciences, University of Leiden, PO Box 9516, 2300 RA Leiden, The Netherlands*

<sup>2</sup>*Division of Population and Evolutionary Biology, School of Biological Sciences, University of Liverpool, Nicholson Building, Brownlow Street, Liverpool L69 3GS, UK*

When local populations are genetically differentiated from one another and partially inbred, as typically occurs in subdivided populations, immigrant genomes are predicted to be at a frequency-dependent fitness advantage due to heterosis (hybrid vigour) in their descendants. We tested this prediction with pedigreed laboratory populations of the butterfly *Bicyclus anynana* and report here on a rapid increase over five generations in the contribution of an initially rare immigrant genome to the local population gene pool. The replicated experimental design, including immigrant controls, demonstrates that the mechanism underlying immigrant genome spread is heterosis, and that the advantage to the immigrant genes is sustained over several generations. Our result suggests that effective migration rates may often be much higher than the numbers of individual migrants assumed by classical population genetics models, with implications for the persistence and evolution of metapopulations.

**Keywords:** inbreeding depression; heterosis; metapopulation; immigrant advantage

## 1. INTRODUCTION

Biologists increasingly accept that natural populations are spatially structured, with habitat loss and fragmentation forcing increasing numbers of species to persist regionally as metapopulations: networks of small local populations connected by migration (Hanski 1999a). Central to the evolution and long-term survival of metapopulations is their ability to sustain genetic variation (Barton & Whitlock 1997), which may depend strongly on the fate of immigrants and their descendants (Keller *et al.* 2001). Population subdivision typically produces some degree of genetic differentiation among, and inbreeding within, local populations, with associated declines in fitness due to inbreeding depression (Charlesworth & Charlesworth 1999). However, only recently (Pamilo *et al.* 1999; Ingvarsson & Whitlock 2000; Whitlock *et al.* 2000) have we begun to explore the dynamic consequences of the interaction between genetic drift, genetic load and migration, and the role of the emergent property of this interaction (heterosis) in shaping genetic variation in metapopulations.

The predominant genetic basis of heterosis is the suppressed expression of deleterious recessive mutations when in the heterozygous state (Crow 1993; Charlesworth & Charlesworth 1999), leading to the prediction that initially rare immigrant genomes are at a fitness advantage because their descendants are more likely, than are those of resident genomes, to be heterozygous for deleterious recessive mutations that cause inbreeding depression in the homozygous state (Ingvarsson & Whitlock 2000; Whitlock *et al.* 2000). Strong selection at loci carrying deleterious recessive alleles is expected to result in rapid changes in the frequencies of whole sections of

chromosomes linked to the loci under selection (i.e. hitchhiking (Maynard Smith & Haigh 1974)). This mechanism would promote genetic mixing among, and genetic variation within, local populations, with the changes being concentrated in chromosomal regions with high densities of deleterious recessive mutations.

The conditions for heterosis-assisted spread of immigrant genomes appear likely to exist in many natural metapopulations: genetic differentiation among local populations is a common feature of many subdivided populations (Whitlock 1992; Dallas *et al.* 1995) and inbreeding depression has now been documented in several natural populations (Keller 1998; Saccheri *et al.* 1998; Coltman *et al.* 1999; Madsen *et al.* 1999; Slate *et al.* 2000). Our experimental design was partly motivated by the metapopulation dynamics of the Glanville fritillary butterfly (*Melitaea cinxia*) in southwestern Finland (Hanski 1999b), where such conditions are known to exist. In this large natural metapopulation consisting of approximately 400 local populations, there is a high risk of local extinction and frequent recolonization of empty patches of suitable habitat. Many new local populations are founded by single gravid females leading to high rates of inbreeding through full-sib mating in the following generation. Furthermore, we have shown that inbreeding depression contributes to local extinction risk in this system (Saccheri *et al.* 1998; Nieminen *et al.* 2001) and that local populations are genetically differentiated from one another (I. J. Saccheri, unpublished data). At the same time, migration between local populations is relatively high (Kuussaari *et al.* 1996).

There are no studies, to our knowledge, of the relative performance of immigrant versus non-immigrant lineages in a natural metapopulation (but see § 3 for comment on a very recently published semi-natural experiment (Ebert *et al.* 2002)). The empirical evidence for heterosis-assisted spread of immigrant genomes comes from two *Drosophila*

\* Author for correspondence (saccheri@liverpool.ac.uk).

*melanogaster* laboratory experiments (Carson 1960; Ball *et al.* 2000) and one field experiment on house mice (Berry *et al.* 1991; Jones *et al.* 1995). In all three experiments, the spread of the immigrant genome was inferred from genetic markers. Carson's fly experiment (Carson 1960) is consistent with the hypothesis that heterosis lends a fitness advantage to unrelated immigrants, but the very similar pattern of equilibrium frequencies at the three marker loci (visible mutants) in reciprocal experiments is suggestive of other forms of selection, acting on the markers themselves, also contributing to the change in allele frequencies. The other, more recent, fly experiment (Ball *et al.* 2000) was inconclusive: there was a fitness advantage to outbred immigrants in their first generation, but no advantage to the  $F_1$  hybrid offspring of inbred immigrants. In the house mouse study (Berry *et al.* 1991; Jones *et al.* 1995), heterosis may have been responsible for the rapid spread of immigrant genomes introduced into an inbred island population, but other plausible mechanisms, such as the superior competitive ability of immigrant males, could not be excluded. The suggestive but inconclusive outcomes of these studies, with respect to heterosis-assisted spread of immigrant genomes, highlights the need for a definitive empirical test.

Our experiment was conducted with the African satyrine butterfly, *Bicyclus anynana*, which has a short generation time and is well suited to laboratory culture. It is also known to carry a high genetic load (Saccheri *et al.* 1996; van Oosterhout *et al.* 2000), predominantly on fertility. We focused on the consequences of a single immigration event between a pair of equally inbred local populations, which could be considered as being a subset of a much larger metapopulation. The experimental treatment consisted transferring a single virgin female from one (donor) inbred local population to another equally inbred, but unrelated, (recipient) local population. The spread of the immigrant's and all the residents' genomes was monitored by keeping track of the pedigree of all individuals in the treatment populations for four generations following the immigration event. In this paper, we describe the relative performance of first-generation immigrants and residents in terms of their contribution to the population gene pool in descendant generations.

## 2. METHODS

### (a) *Inbred local populations*

Six 'newly inbred' laboratory lines (local populations) were established with single pairs of mating butterflies taken from a large outbred laboratory stock population. Two additional, 'highly inbred' lines were included to explore the prediction that among local populations for which a long history of inbreeding may have led to partial purging of the genetic load, there should be weaker heterosis and hence less of a fitness advantage to a rare immigrant genome. The two highly inbred lines (A and B) were the product of six generations of continuously small (three pairs) adult population size, from a previous experiment (van Oosterhout *et al.* 2000), followed by three rounds of single-pair bottlenecks with intervening recovery periods. After the final single-pair bottleneck imposed at the start of this experiment, A and B were estimated to have an average inbreeding coefficient of *ca.* 0.8. These lines were both characterized by low egg-hatching rates and low mating frequency. In the  $F_2$  generation

of the six newly inbred lines, corresponding to the generation when the initial immigration events took place, all individuals had an inbreeding coefficient of 0.25. Due to inbreeding depression, 20–50% of the matings in any population produced sterile clutches.

### (b) *Experimental design and procedures*

Two generations following this founding event (i.e.  $F_2$ , constituting the first generation of inbred individuals for the newly inbred lines), the six newly inbred lines were randomly paired into recipient and donor local populations (resulting in three newly inbred recipient–donor pairs). The two highly inbred lines were treated in the same way to form a fourth recipient–donor pair. Experimental populations were established from 29 purebred families (i.e. the offspring from matings between resident males and females) and one hybrid family (from a mating between a resident male and a donor/immigrant female). For each recipient–donor pair, two replicate treatment populations were constructed using different purebred and hybrid families (e.g. 1(1) and 1(2)). A third, control, population was set up for each recipient–donor pair in which the hybrid family was replaced by a 30th purebred family from the same population. In this and all subsequent generations, adult local population size was kept at 30 mating pairs.

The butterflies were reared as individual families from egg to the adult stage. Larvae were fed on potted maize plants in net cages and adults were fed on moist banana. Family size varied considerably (range, 0–83; mean, 17 hatching larvae). Density-dependent effects on larval viability among families were minimized by limiting the number of larvae per cage to a maximum of 20 (fourth and fifth instars), using additional enclosures for families with more larvae. Male and female adults were numbered (by family), allowing the pedigree of all individuals to be traced, and placed into single-sex cages on the day of emergence. After the majority of butterflies from a population had emerged, mating cages were composed of 60 virgin females and 60 virgin males (maximally 8 days from the day of emergence of the first adult, giving a modal age at mating of 3 to 4 days), each family's contribution being in proportion to its size (assessed at the pupal stage). The first 30 matings were used as parents for the next generation. This procedure allowed all males equal opportunity to mate with any female within a population, and for selection on mating behaviour to operate. Mating pairs were isolated while *in copula* and eggs subsequently collected from the female for a fixed period of 24 h.

### (c) *Analysis*

Pedigrees were drawn using PEDDRAW (Mamelka *et al.* 1993). The genetic contribution of each founder to the population gene pool (the founder genome representation) in each generation was calculated from the pedigrees, assuming that individuals inherit each of their grandparental genomes in equal parts (one-quarter). In reality, recombination and independent assortment of chromosomes generate variation around this expectation such that grandparental contributions will not be exactly equal. Thus, ancestry calculated from a pedigree is not a perfect predictor of the proportion of genes inherited from a particular ancestor. In the present context of estimating the proportion of the immigrant genome in descendants, the expectation is a conservative assumption because selection should favour those individuals that carry relatively more of the immigrant genome and as a result show more heterosis. Relative founder representation was calculated as the ratio of immigrant founder genome represen-

tation and the median founder genome representation for that population in each generation.

A generalized linear regression model was used to assess the correlation, in generations three and four, between individual coefficients of inbreeding, calculated from the pedigrees using the Pedsys program KINSHIP (Dyke & Freeman-Shade 1999), and the proportion of the immigrant genome carried by individuals (also calculated from the pedigrees). *F*-tests were based on the ratio of the mean square of the common regression across all experimental lines (after fitting different intercepts for each line) and the mean square describing the variance in regression slopes among lines.

### 3. RESULTS AND DISCUSSION

A graphical illustration of the typical spread of the immigrant genomes is provided by a representative pedigree (figure 1). This pedigree shows that the immigrant genome, initially present in only one out of 32 reproductively successful individuals (the other 14 mating pairs produced no adult offspring, which was largely an effect of inbreeding depression), spread rapidly in subsequent generations, such that four generations after the immigration event, all individuals in the population had some immigrant ancestry. However, large variance in survival among founder genomes and rapid coalescence of lineages is in any case expected in these small populations subject to large sampling variance.

To answer the question of whether the immigrant genomes performed significantly better than the resident genomes, we used the pedigrees to calculate a measure of reproductive success for all founders (residents and immigrants) that left adult offspring (i.e. excluding those founding pairs that produced sterile clutches or very few hatching larvae). This is the founder genome representation: the proportion of the gene pool in a given generation that originates from a specific founder. The distribution of founder genome representation in the fifth generation (figure 2) is highly skewed, with an average of 36% of founders making no contribution, but with the genomes of very few founders representing from 10–25% of the gene pool. In all cases, the immigrant genome was the most, or in one case (1(1)) second-most, successful founding genome.

Figure 2 shows unequivocally that within four generations the cumulative reproductive success of the immigrants was significantly greater than that of their resident counterparts. But what do the temporal dynamics of immigrant genome spread look like? The relative contribution of each generation to the immigrant founder genome representation in the fifth generation is shown in figure 3. Because in *B. anynana* inbreeding depression is greater for fertility than viability (Saccheri *et al.* 1996; van Oosterhout *et al.* 2000), we expected that the greatest change would occur between the second and third generations as a result of improved fertility of  $F_1$  hybrids, with an inbreeding coefficient of zero. There is evidence for this effect in two lines (1(1), 1(2)), but overall the *relative* change between the first and second generations is not significantly smaller than between the second and third generations. This better than expected performance between the first and second generations was probably due to some heterosis for zygote viability and mating success

in  $F_1$  hybrids, but may also reflect insufficient statistical power.

Large increases in (immigrant) relative founder representation also occurred for some lines in subsequent generations. This must partly be due to the immigrant genome continuing to be carried by relatively less inbred individuals. In the third generation, the correlation between individual coefficients of inbreeding and the proportion of the genome that was of immigrant origin was strongly negative in seven out of eight lines and significant overall ( $p < 0.01$ ), and in the fourth generation six lines showed a negative correlation, but this was not significant overall.

Figure 3 also shows that the largest founding families in the non-immigrant control populations (not significantly different in size from first-generation immigrant families) either achieved minimal increases in relative founder representation by the fifth generation or, in the case of the highly inbred control, actually became extinct. Comparison with the performance of the immigrant families indicates that the size of founding families alone has little influence on the founder genome representation in later generations and is much less important than immigrant ancestry, confirming the hypothesis that heterosis is driving the spread of the immigrant genomes. The magnitude of the effect varies considerably among lines but (with the one exception of the highly inbred line A(1)) is very substantial. In the fifth generation, the performance of first-generation immigrant genomes is 2–71 (mean, 22) times better than the average non-immigrant founder genome. By contrast, the average performance of the largest founding families in (non-immigrant) control populations was only twice the median performance.

One explanation for the relatively poor performance of the immigrant genome in one of the highly inbred lines (A(1)) is that the recipient population had been purged of deleterious recessives. However, strong heterosis in a large sample of  $A \times B$  hybrid offspring, and the very good performance of the immigrant genome in the replicate A(2), indicate that this was not the case. The true explanation has more to do with the conspicuously low hatching rate of eggs produced by matings involving  $F_1$  hybrid offspring in A(1), the cause of which is currently unknown. This result suggests that, because of the difficulty of eliminating the alleles underlying inbreeding depression (Hedrick 1994; Lynch *et al.* 1995; Saccheri *et al.* 1998), frequent close inbreeding cannot be assumed to eliminate the benefits of heterosis to immigrant genomes.

Immigration is known to play a crucial role in the persistence of small and isolated populations through its direct impact on population size: the (demographic) 'rescue effect' (Brown & Kodric-Brown 1977). The heterosis-assisted spread of immigrant genomes shown here may further reduce the extinction risk of individual local populations and metapopulations by ameliorating inbreeding depression and enhancing adaptive genetic variability (Spielman & Frankham 1992; Madsen *et al.* 1999; Richards 2000). This 'genetic rescue effect', expressed through increased birth rates and lower mortality, represents an additional mechanism through which immigrants may benefit small and fragmented populations. Through hitchhiking, the same process may also assist the spread of traits associated with migrant genotypes, such as dispersal

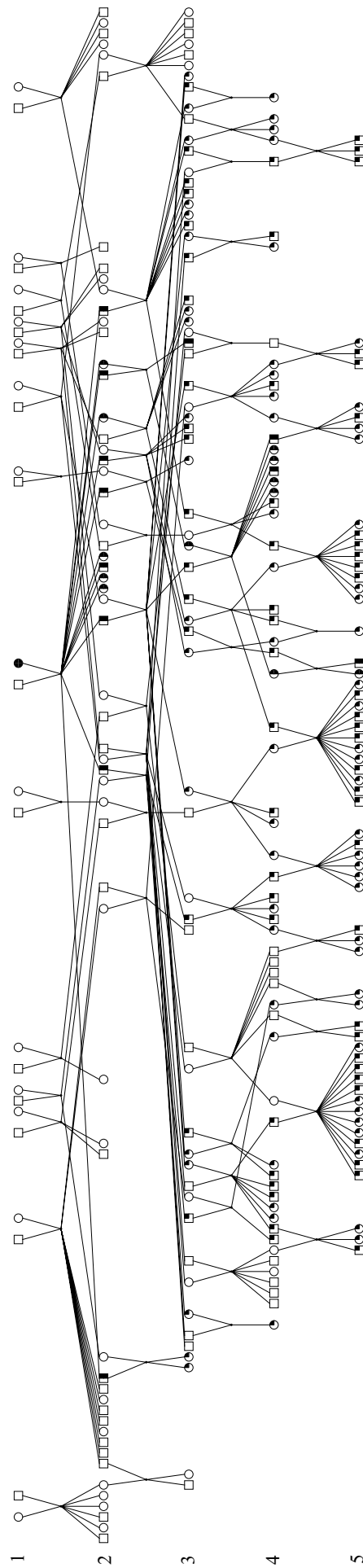


Figure 1. Five-generation pedigree of one of the experimental populations (3(1)) highlighting (in black) the proportion of immigrant genome in the first-generation immigrant female and her descendants. Circles represent females and squares males, with half-filled and quarter-filled symbols representing immigrant genome proportions of 0.25–0.5 and 0–0.25, respectively. Only those founding pairs that produced mating grandoffspring are shown in this pedigree. Generation numbers are shown on the left.

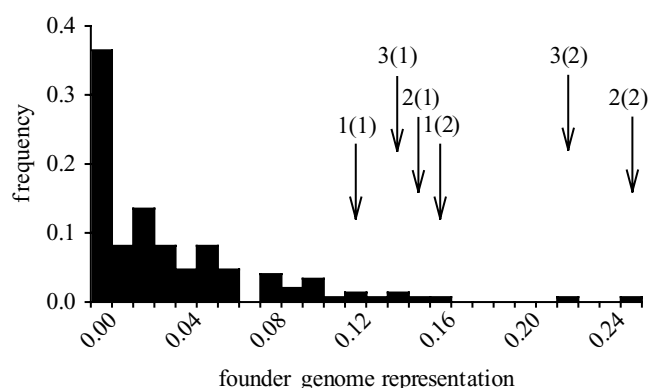


Figure 2. Distribution of founder genome representation in the fifth generation. Distributions for the nine newly inbred lines (including controls) were very similar and therefore combined into a single histogram. The arrows indicate the founder genome representation of the immigrant genome in each of the six newly inbred treatment lines. Only those founders that left adult F<sub>1</sub> offspring are included. The distributions of founder genome representation for the highly inbred lines (not shown) were broadly similar, but the relative success of the immigrant genome differed among the two replicates: one showing average performance, while the other had the highest representation by the fifth generation.

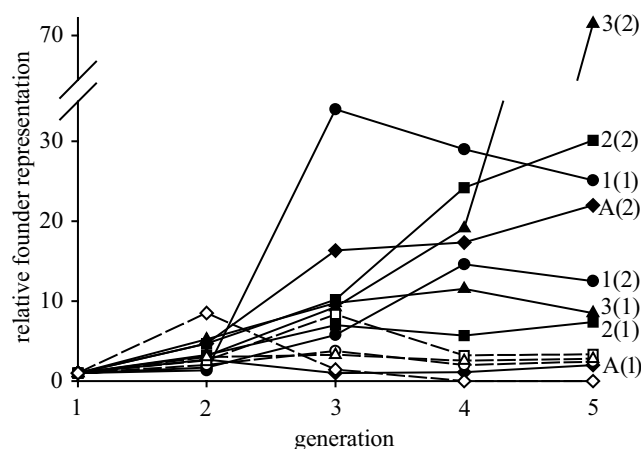


Figure 3. Change through time of immigrant founder genome representation relative to non-immigrant founder genomes (relative founder representation). The filled symbols correspond to the values for each of the two replicates in four recipient–donor population pairs. Numbers outside and inside parentheses indicate, respectively, the recipient–donor line cross (A refers to highly inbred lines) and replicate. The corresponding open symbols give the relative founder representation for the founding female with the largest family in the relevant non-immigrant control populations.

propensity itself (Heino & Hanski 2001). Conversely, it may retard the process of local adaptation. In any case, the pattern of immigrant genome introgression is likely to be highly non-random and clustered around regions associated with strong heterotic effects.

In this experiment, the spread of the immigrant genomes was strikingly fast. In natural metapopulations the effect is likely to be more subtle, in part because local populations are usually more weakly differentiated (and the process itself is expected to reduce differentiation).

Furthermore, immigrants may be disadvantaged for other reasons, such as the reproductive costs of migration or local maladaptedness. Conversely, heterosis is generally stronger under conditions of greater environmental stress (Bijlsma *et al.* 1999), disease (Coltman *et al.* 1999) or competition (Meagher *et al.* 2000). Quantifying the spread of immigrant genomes in a range of natural metapopulations remains a major challenge for evolutionary ecologists, but there is growing evidence that the underlying mechanisms—inbreeding depression and heterosis—are widespread in natural populations (Crnokrak & Roff 1999; Rossiter *et al.* 2001), even at low levels of inbreeding (Amos *et al.* 2001). Our results suggest that genetic subdivision of populations, most commonly through habitat fragmentation, tends to favour migrant genomes (that have overcome the initial costs of migration itself). As a consequence of this effect and the underlying mechanism, the impact of migration on fitness, effective population size, evolutionary change and patterns of intra- and inter-genomic variation may be much greater than assumed by current models.

A recently published experiment (Ebert *et al.* 2002) using *Daphnia magna* reports on a large fitness advantage to asexually reproducing F<sub>1</sub> hybrids produced after the artificial introduction of immigrant clones into inbred demes. Although the study cannot be said to document natural immigration events (also because the competition experiments were initiated with 200–300 residents and immigrants in equal numbers), it demonstrates that strong heterosis may be achieved under natural conditions in crosses between genotypes occurring naturally in the same metapopulation and that hybrids outcompete inbred parental strains. By contrast, the significant contribution of our study, conducted on a very different organism and starting with a single immigrant individual per deme, is to show that the advantage to immigrant genes may be sustained over several generations; using pedigrees, rather than a few marker loci, to describe the details of how the spread of immigrant ancestry through the population may unfold. In their article on the *Daphnia* study, Ives & Whitlock (2002) suggest that even in populations that do not show classical metapopulation dynamics, extinction and colonization processes may nevertheless generate appreciable genetic structuring. This needs to be verified empirically but would open up further the opportunity for heterosis-assisted spread of immigrant genes in natural populations.

We gratefully acknowledge the technical assistance of R. Stam, E. Schlatmann and B. de Winter; the valuable comments of M. Begon, D. Ebert, C. Haag, I. Hanski, R. Nichols and S. Sait and the financial support of the European Union TMR Network ‘Fragland’ (contract no. ERBFMRXCT980227). Thanks to M. Whitlock and an anonymous referee for additional comments.

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