

# Microsatellite diversity predicts recruitment of sibling great reed warblers

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Inbreeding increases the level of homozygosity, which in turn might depress fitness. In addition, individuals having the same inbreeding coefficient (e.g. siblings) vary in homozygosity. The potential fitness effects of variation in homozygosity that is unrelated to the inbreeding coefficient have seldom been examined. Here, we present evidence from wild birds that genetic variation at five microsatellite loci predicts the recruitment success of siblings. Dyads of full-sibling great reed warblers (*Acrocephalus arundinaceus*), one individual of which became a recruit to the natal population while the other did not return, were selected for the analysis. Each dyad was matched for sex and size. Local recruitment is strongly tied to fitness in great reed warblers as the majority of offspring die before adulthood, philopatry predominates among surviving individuals and emigrants have lower lifetime fitness. Paired tests showed that recruited individuals had higher individual heterozygosity and higher genetic diversity, which was measured as the mean squared distance between microsatellite alleles (mean  $d^2$ ), than their non-recruited siblings. These relationships suggest that the microsatellite markers, which are generally assumed to be neutral, cosegregated with genes exhibiting genetic variation for fitness.

**Keywords:** great reed warbler; microsatellite; homozygosity; mean  $d^2$ ; recruitment; fitness

## 1. INTRODUCTION

Homozygosity leads to the expression of recessive deleterious alleles and loss of heterozygous advantage (Hartl & Clark 1997; Lynch & Walsh 1998) and might cause inbreeding depression, i.e. the often-found negative relationship between the inbreeding coefficient and fitness (Price & Waser 1979; Van Noordwijk & Scharloo 1981; Dudash 1990; Jiménez *et al.* 1994).

In recent years, inbreeding coefficients have commonly been inferred from the allele frequency data of neutral markers and several studies have found a relationship between genetic variation and fitness (Bensch *et al.* 1994; Coltman *et al.* 1998; Coulson *et al.* 1998; Saccheri *et al.* 1998; Slate *et al.* 2000). However, due to the random assortment of parental chromosomes, genetic variation within and between parents might establish variation in the level and distribution of homozygosity within a group of siblings, i.e. individuals of the same inbreeding coefficient. Such variation between siblings at a specific locus is expected when the parents share one or two identical alleles and at least one of the parents is not a homozygote. In accordance with inbreeding theory, there is a potential relationship between fitness and variation in homozygosity that is unrelated to the inbreeding coefficient. To our knowledge, this hypothesis has never been explicitly tested in the wild, although it has been supported by a recent study of flat oysters (*Ostrea edulis*) where experimental crosses of full-sibs resulted in a higher than expected frequency of offspring being heterozygous at four microsatellite loci (Bierne *et al.* 1998) and by recent findings of heterozygote advantage at particular coding loci, for example at the major histocompatibility complex in humans (Thursz *et al.* 1997; Carrington *et al.* 1999).

In the present paper, we used data from a long-term population study of great reed warblers (Bensch &

Hasselquist 1991; Hasselquist *et al.* 1995, 1996; Bensch 1996; Bensch *et al.* 1998; Hasselquist 1998; Hansson *et al.* 2000a) for evaluating whether genetic variation at five highly polymorphic microsatellite loci might predict an early-expressed fitness trait of individuals of the same inbreeding coefficient. We selected dyads of full-siblings for the test, one individual of which became a recruit to the natal population while the other did not return. Sex and size were matched within each dyad. Since microsatellites are supposed to be selectively neutral (Queller *et al.* 1993; Jarne & Lagoda 1996), any correlation between fitness and heterozygosity suggests that the markers cosegregate with genes that are under selection (Houle 1989; Hartl & Clark 1997; Lynch & Walsh 1998). We calculated two different measures of individual genetic diversity from the microsatellite data: individual heterozygosity and the mean  $d^2$ , i.e. the mean squared distance between microsatellite alleles (Coulson *et al.* 1998; Pemberton *et al.* 1999). These two measures score heterozygotes differently and have been, under the assumption of a stepwise mutation process of microsatellites (Goldstein *et al.* 1995), suggested to put emphasis on different ends of the inbreeding–outbreeding continuum (Coulson *et al.* 1998; Pemberton *et al.* 1999). If the microsatellite loci examined cosegregate with genes affecting fitness, either due to homozygosity at deleterious recessive loci or due to over-dominance, we expect the returning individual to have higher values of individual genetic diversity than its non-returning sibling.

## 2. MATERIAL AND METHODS

The great reed warbler has a facultative, polygynous, social mating system (Dyrce 1986; Hasselquist 1998). It breeds in productive lakes and marshes over large parts of the Palaearctic and winters in tropical Africa (Cramp 1992). The species colonized the Scandinavian peninsula in the 1960s and in 1999 *ca.* 85% of the *ca.* 450 Scandinavian males held territories in 12 larger

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Table 1. *Numbers of alleles, size ranges of alleles, average heterozygosities and  $d^2$  ( $\log_{10}$  transformed) (see § 2) of recruited and non-recruited great reed warbler siblings ( $n = 50$  pairs) at five microsatellite loci*

(The results from pairwise tests at each locus are also shown.)

locus	number of alleles	size range (bp)	heterozygosity				$d^2$			
			recruited	non-recruited	$z^a$	$p$	recruited	non-recruited	$z^a$	$p$
Aar3	15	190–254	0.78	0.70	0.89	0.371	1.35	1.16	1.07	0.287
Aar4	6	112–124	0.56	0.52	0.63	0.527	0.33	0.35	−0.50	0.618
Aar5	10	75–97	0.90	0.90	0.00	1.000	0.92	0.93	−0.04	0.971
Ppi2	17	238–286	1.00	0.88	2.45	0.014	1.66	1.22	3.40	0.001
G61 <sup>b</sup>	7	136–162	0.76	0.56	1.90	0.059	0.52	0.44	1.68	0.094

<sup>a</sup>Wilcoxon signed-ranks test.<sup>b</sup>Males only ( $n = 25$  sibling pairs) (locus located on the Z chromosome).

subpopulations (B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results; see also Hasselquist 1995). The great reed warbler population at Lake Kvismaren, southern Central Sweden (59°10' N, 15°25' E), was established by a few individuals in 1978, which was followed by a gradual increase in numbers until 1988. Since 1988, the population has remained relatively stable with *ca.* 60 breeding birds per year (Hansson *et al.* 2000b). We studied the breeding ecology of the birds in this population between 1983 and 2000 (Bensch & Hasselquist 1991; Bensch 1996; Hasselquist 1998; Hansson *et al.* 2000a). All adults in the population have been ringed since 1985 with individually unique combinations of one aluminium and several coloured plastic rings and their pairing and reproductive success have been measured. When nestlings were nine days old, we measured body mass and the length of their innermost primary flight feather and ringed them. We have taken blood samples from all individuals examined since 1987 and isolated DNA with phenol/chloroform-isoamylalcohol extraction (Bensch *et al.* 1994; Westerdahl *et al.* 1997). In addition, at the nearest breeding site, Lake Segersjö (12 km east of Lake Kvismaren), most breeding birds and many nestlings were ringed between 1987 and 2000 (B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results).

We have found low levels of genetic variation in the great reed warbler population at Lake Kvismaren in previous studies (Bensch *et al.* 1994; Bensch & Hasselquist 1999; Hansson *et al.* 2000b), which is probably the result of the recent founder event (Hansson *et al.* 2000b). A negative correlation between the multi-locus DNA fingerprinting similarity of pair mates and egg hatchability has indicated the occurrence of inbreeding depression in the population (Bensch *et al.* 1994). Despite being a long-distance migrant, the birds show a high degree of philopatry to our study site and the majority of birds that do disperse settle at nearby breeding sites (Hasselquist 1995; Bensch *et al.* 1998; B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results).

The recruitment of nestling great reed warblers in the present study was measured in the Kvismare–Segersjö area. This measure of recruitment is a good estimate of viability for three reasons.

(i) A high proportion of the young in most passerines die during their first year, i.e. from the nestling stage to their first spring (see e.g. Wheelwright & Mauck 1998). We have previously estimated that more than 70% of great reed warbler nestlings die during this period (Hasselquist 1995; Bensch *et al.* 1998; B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results).

(ii) A high proportion of the surviving offspring are philopatric (50–60%) (Hasselquist 1995; Bensch *et al.* 1998; B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results).

(iii) We have previously shown that dispersing males suffer from a significant fitness reduction (Bensch *et al.* 1998). This is also true for females, albeit not significantly so (Bensch *et al.* 1998).

We selected 50 sibling dyads from the 1987–1996 cohorts for analysis. We confirmed that siblings within dyads had the same genetic parents using molecular methods (Hasselquist *et al.* 1995, 1996; D. Hasselquist, D. Arlt, S. Bensch and B. Hansson, unpublished results). The siblings were matched for sex (as assessed by molecular sexing) (Westerdahl *et al.* 1997, 2000), size (length of the innermost primary feather) and body mass. Paired tests confirmed that there was no significant difference in size and body mass between recruited and non-recruited siblings (primary feather length  $t_{49} = -0.56$  and  $p = 0.58$  and mass  $t_{49} = -0.83$  and  $p = 0.41$ ). Male siblings were typed for allelic variation at five microsatellite loci (table 1). The source of loci Aar3, Aar4 and Aar5 was Hansson *et al.* (2000b), that of locus Ppi2 was Martinez *et al.* (1999) and that of locus G61 was Nishiumi *et al.* (1996). These microsatellites consist of dinucleotide repeat units and are highly polymorphic in great reed warblers (Hansson *et al.* 2000b). The G61 locus is located on the sex-specific Z chromosome, which means that females have one copy only. Hence, females were not scored at this locus. The primer sequences and amplification conditions are given in Hansson *et al.* (2000b).

Individual heterozygosity was calculated as the number of heterozygous loci (scored as unity for each heterozygous locus and zero for each homozygous locus) divided by the number of loci examined. The value of  $d^2$  at the  $n$ th locus was calculated for each individual as the squared difference in the number of repeat units between the two alleles, i.e.  $d^2 = (\text{allele}_a - \text{allele}_b)^2$ . The distribution of  $d^2$  was highly skewed at all loci (data not shown). In order to improve normality, we  $\log_{10}$  transformed  $d^2$  ( $\log_{10}(d^2 + 1)$ ) (Sokal & Rohlf 1995) and subsequent analyses were performed on the transformed values. The mean  $d^2$  is the average of  $d^2$  over all investigated loci in an individual (Coulson *et al.* 1998; Pemberton *et al.* 1999).

We tested differences between siblings with two-tailed, paired, parametric tests. We generated residuals from general linear models with brood and recruitment/non-recruitment as independent

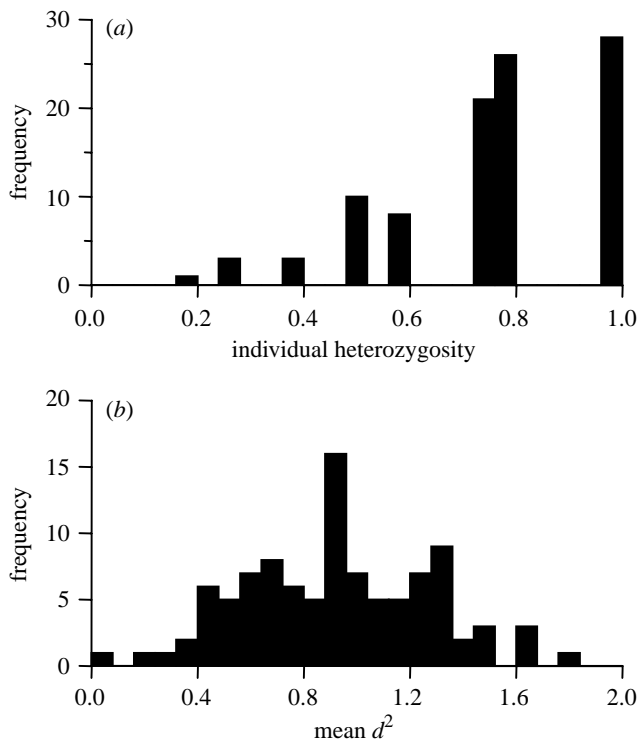


Figure 1. Frequency distribution of (a) individual heterozygosity and (b) the mean  $d^2$  of 100 great reed warbler siblings born between 1987 and 1996 and genotyped at four (females) and five (males) microsatellite loci. The mean  $d^2$  is based on  $\log_{10}$ -transformed  $d^2$  (see § 2).

categorical variables and checked them for normality using the Kolmogorov–Smirnov test with Lilliefors' correction (Sokal & Rohlf 1995). When the assumption of normality was violated, two-tailed, paired, non-parametric statistics were used (Wilcoxon signed-ranks test) (Siegel & Castellan 1989).

### 3. RESULTS

Histograms of the genetic data showed that individual heterozygosity was skewed, whereas the mean of  $d^2$  (based on the  $\log_{10}$ -transformed  $d^2$ ) (see § 2) was approximately normally distributed (figure 1). As might be expected from the fact that homozygotes contribute to both measures of genetic diversity, we found that individual heterozygosity and the mean  $d^2$  were positively correlated ( $r_s = 0.50$ ,  $n = 100$  and  $p < 0.001$ ). Paired tests showed that the recruited sibling had higher individual heterozygosity ( $z = 2.28$ ,  $n = 50$  and  $p = 0.023$ ) and a higher mean  $d^2$  ( $t_{49} = 2.26$  and  $p = 0.028$ ) than the non-recruited sibling. The average differences ( $\pm$ s.e.) in individual heterozygosity and the mean  $d^2$  between siblings in dyads are shown in figure 2. When we analysed each locus separately, both heterozygosity ( $p = 0.014$ ) and  $d^2$  ( $p = 0.001$ ) were significantly higher for the recruited sibling at the Ppi2 locus and a statistical trend for the same pattern was found at the G61 locus ( $p = 0.059$  and  $p = 0.094$ , respectively) (table 1).

### 4. DISCUSSION

Our major and novel finding was that local recruitment of sibling great reed warblers was significantly

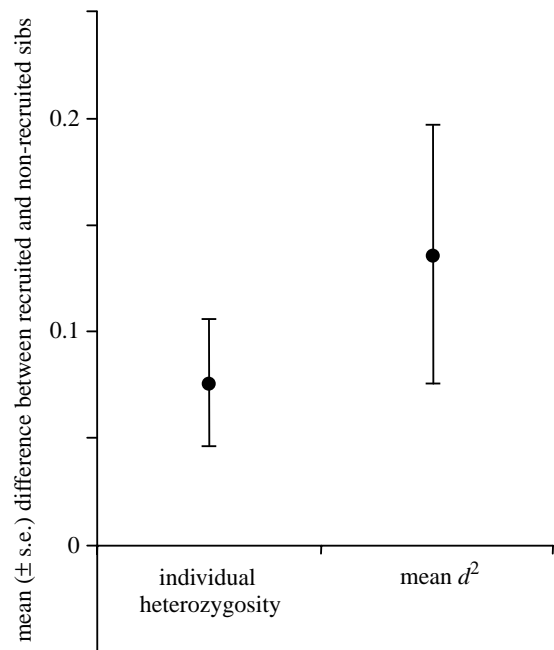


Figure 2. Mean difference ( $\pm$ s.e.) in individual heterozygosity (left) and the mean  $d^2$  (right) between recruited and non-recruited great reed warbler siblings. Recruited individuals scored both higher individual heterozygosity and a higher mean  $d^2$  than their non-recruited siblings. The mean  $d^2$  is based on  $\log_{10}$ -transformed  $d^2$  (see § 2).

associated with high allelic diversity at five polymorphic microsatellite loci. Local recruitment was highly related to survival over the period between fledging and adulthood in both sexes of great reed warbler (Hasselquist 1995; Bensch *et al.* 1998; B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results), as well as to reproductive success in males (Bensch *et al.* 1998). Hence, to the authors' knowledge, this is the first study on wild animals to demonstrate that a difference in the level of homozygosity at supposedly neutral markers between individuals of the same inbreeding coefficient (siblings) predicts an early-expressed fitness trait.

Microsatellite loci are not thought to be under selection (Queller *et al.* 1993; Jarne & Lagoda 1996). This suggests that the markers used in the present study cosegregated with and, hence, provided information about other polymorphic genes that are important for fitness (Houle 1989; Hartl & Clark 1997; Lynch & Walsh 1998) as, for example, has been shown for microsatellites within the major histocompatibility complex in Soay sheep (*Ovis aries*) (Paterson *et al.* 1998). The probability that a marker provides information about one or more important loci will increase with increasing levels of linkage disequilibrium (Houle 1989; Hartl & Clark 1997; Lynch & Walsh 1998). The particularly high levels of linkage disequilibrium in the study of Bierne *et al.* (1998) on flat oysters resulted from full-sibs being crossed in the experiment and they also found a strong relationship between individual heterozygosity and survival within crosses. The pedigree data from our long-term great reed warbler study suggested that incestuous inbreeding (between cousins or higher order relatives) occasionally occurs in the population (Bensch *et al.* 1994) but none of the dyads of siblings included in this study stemmed from such matings.

However, Swedish great reed warblers exhibit other demographic properties that generally create and promote linkage disequilibrium (Houle 1989; Hästbacka *et al.* 1992; Hartl & Clark 1997; De la Chapelle & Wright 1998; Lynch & Walsh 1998). First, the population has expanded rapidly since the founder event in the 1960s (Hansson *et al.* 2000b). Second, local populations are small and patchily distributed (Hasselquist 1995; Hansson *et al.* 2000b) and there is restricted dispersal between populations (Hasselquist 1995; Bensch *et al.* 1998; B. Hansson, S. Bensch, D. Hasselquist and B. Nielsen, unpublished results). Third, the presence of two distinct mitochondrial DNA lineages in the region, representing western and eastern European haplotypes, respectively (Bensch & Hasselquist 1999), suggests mixing of birds originating from populations with different gene frequencies. Hence, it is possible that moderate to high levels of linkage disequilibrium were present in our study population and that each particular marker provided information about a relatively large part of the chromosome. An alternative explanation is that the markers we used happen to be tightly linked to single fitness-associated genes. Even if the latter explanation may in theory apply for the association between the mean  $d^2$  and fitness (Coulson *et al.* 1998; Pemberton *et al.* 1999), it is less likely to explain the relationship between individual heterozygosity and fitness. This is because most microsatellite loci mutate at higher rates than other genes (Queller *et al.* 1993; Jarne & Lagoda 1996) making many individuals heterozygous at the microsatellites despite being homozygous at single, less-mutable, linked genes.

Recent studies of harbour seals (*Phoca vitulina*) and red deers (*Cervus elaphus*) have shown that their mean  $d^2$  but not individual heterozygosities were related to juvenile survival (Coltman *et al.* 1998; Coulson *et al.* 1998; see also Pemberton *et al.* 1999). On the other hand, in our population of great reed warblers both individual heterozygosity and the mean  $d^2$  were associated with juvenile survival. We suggest two possible explanations for the difference between these studies. First, the mean  $d^2$  only provides information about the coalescence time of microsatellite alleles for loci mutating according to the stepwise mutation process (Goldstein *et al.* 1995; Coulson *et al.* 1998). Not all microsatellites seem to obey a strict stepwise mutation process (Estoup *et al.* 1995; Primmer *et al.* 1998). If the microsatellites examined in the great reed warblers frequently mutate in an irregular fashion, there will be no clear relationship between their mean  $d^2$  and the relatedness of alleles. Consequently, the mean  $d^2$  will not provide better resolution of the degree of outcrossing of individuals than just a flat estimate of heterozygosity. Second, according to the stepwise mutation process, the mean  $d^2$  is hypothesized to detect inbreeding events deeper in the pedigree than individual heterozygosity (Coulson *et al.* 1998; Pemberton *et al.* 1999). Hence, differences in the demographic history and current dispersal levels of the populations might explain differences in the relationship between fitness traits and the two measures of genetic diversity. Coltman *et al.* (1998) and Coulson *et al.* (1998) suggested that intermixing of individuals originating from populations that had been isolated for a considerable time resulted in offspring showing both a heterosis effect and a high mean  $d^2$ . However, intermixing of individuals of genetically different origin also occurs at

our study site, as suggested by the presence of two distinct mitochondrial DNA lineages (Bensch & Hasselquist 1999), a moderate level of inflow of novel microsatellite alleles in recent years and an increase in genetic variation in the population over time (Hansson *et al.* 2000b). Despite this similarity, it might be that the great reed warbler population differs from the deer and seal populations in its level of isolation, the regularity with which individuals disperse between populations or other demographic aspects that might affect the fitness consequences of matings between individuals of recent and remote ancestry.

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## REFERENCES

- Bensch, S. 1996 Female mating status and reproductive success in the great reed warbler: is there a potential cost of polygyny that requires compensation? *J. Anim. Ecol.* **65**, 283–296.
- Bensch, S. & Hasselquist, D. 1991 Territory infidelity in the polygynous great reed warbler *Acrocephalus arundinaceus*: the effect of variation in territory attractiveness. *J. Anim. Ecol.* **60**, 857–871.
- Bensch, S. & Hasselquist, D. 1999 Phylogeographic population structure of great reed warblers: an analysis of mtDNA control region sequences. *Biol. J. Linn. Soc.* **66**, 171–185.
- Bensch, S., Hasselquist, D. & Von Schantz, T. 1994 Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler? *Evolution* **48**, 317–326.
- Bensch, S., Hasselquist, D., Nielsen, B. & Hansson, B. 1998 Higher fitness for philopatric than for immigrant males in a semi-isolated population of great reed warblers. *Evolution* **52**, 877–883.
- Bierne, N., Launey, S., Naciri-Graven, Y. & Bonhomme, F. 1998 Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics* **148**, 1893–1906.
- Carrington, M., Nelson, G. W., Martin, M. P., Kissner, T., Vlahov, D., Goedert, J. J., Kaslow, R., Buchbinder, S., Hoots, K. & O'Brien, S. J. 1999 HLA and HIV-1: heterozygote advantage and B\*35–Cw\*04 disadvantage. *Science* **283**, 1748–1752.
- Coltman, D. W., Bowen, W. D. & Wright, J. M. 1998 Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proc. R. Soc. Lond.* **B 265**, 803–809.
- Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. 1998 Microsatellites reveal heterosis in red deer. *Proc. R. Soc. Lond.* **B 265**, 489–495.
- Cramp, S. 1992 *Handbook of the birds of Europe, the Middle East and North Africa*, vol. VI. New York: Oxford University Press.
- De la Chapelle, A. & Wright, F. A. 1998 Linkage disequilibrium mapping in isolated populations: the example of Finland revisited. *Proc. Natl Acad. Sci. USA* **95**, 12 416–12 423.
- Dudash, M. R. 1990 Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* **44**, 1129–1139.

- Dyrce, A. 1986 Factors affecting facultative polygyny and breeding results in the great reed warbler (*Acrocephalus arundinaceus*). *J. Ornithol.* **127**, 447–461.
- Estoup, A., Garnery, L., Solignac, M. & Cornuet, J.-M. 1995 Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics* **140**, 679–695.
- Goldstein, D. B., Linares, A. R., Cavalli-Sforza, L. L. & Feldman, M. W. 1995 An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463–471.
- Hansson, B., Bensch, S. & Hasselquist, D. 2000a Patterns of nest predation contribute to polygyny in the great reed warbler. *Ecology* **81**, 319–328.
- Hansson, B., Bensch, S., Hasselquist, D., Lillandt, B.-G., Wennerberg, L. & Von Schantz, T. 2000b Increase of genetic variation over time in a recently founded population of great reed warblers (*Acrocephalus arundinaceus*) revealed by microsatellites and DNA fingerprinting. *Mol. Ecol.* **9**, 1529–1538.
- Hartl, D. L. & Clark, A. G. 1997 *Principles of population genetics*, 3rd edn. Sunderland, MA: Sinauer Associates, Inc.
- Hasselquist, D. 1995 Demography and lifetime reproductive success in the polygynous great reed warbler. *Jap. J. Ornithol.* **44**, 181–194.
- Hasselquist, D. 1998 Polygyny in the great reed warbler: a long-term study of factors contributing to male fitness. *Ecology* **79**, 2376–2390.
- Hasselquist, D., Bensch, S. & Von Schantz, T. 1995 Low frequency of extra-pair paternity in the polygynous great reed warbler. *Behav. Ecol.* **6**, 27–38.
- Hasselquist, D., Bensch, S. & Von Schantz, T. 1996 Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* **381**, 229–232.
- Hästbacka, J., De la Chapelle, A., Kaitila, I., Sistonen, P., Weaver, A. & Lander, E. 1992 Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. *Nature Genet.* **2**, 204–211.
- Houle, D. 1989 Allozyme-associated heterosis in *Drosophila melanogaster*. *Genetics* **123**, 789–801.
- Jarne, P. & Lagoda, P. J. L. 1996 Microsatellites, from molecules to populations and back. *Trends Ecol. Evol.* **11**, 424–429.
- Jiménez, J. A., Hughes, K. A., Alaks, G., Graham, L. & Lacy, R. C. 1994 An experimental study of inbreeding depression in a natural habitat. *Science* **266**, 271–273.
- Lynch, M. & Walsh, B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer Associates, Inc.
- Martinez, J. G., Soler, J. J., Soler, M., Møller, A. P. & Burke, T. 1999 Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution* **53**, 269–278.
- Nishiumi, I., Yamagishi, S., Maekawa, H. & Shimoda, C. 1996 Paternal expenditure is related to brood sex ratio in polygynous great reed warblers. *Behav. Ecol. Sociobiol.* **39**, 211–217.
- Paterson, S., Wilson, K. & Pemberton, J. M. 1998 Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proc. Natl Acad. Sci. USA* **95**, 3714–3719.
- Pemberton, J. M., Coltman, D. W., Coulson, J. C. & Slate, J. 1999 Using microsatellites to measure the fitness consequences of inbreeding and outbreeding. In *Microsatellites: evolution and applications* (ed. D. B. Goldstein & C. Schlötterer), pp. 151–164. Oxford University Press.
- Price, M. V. & Waser, N. M. 1979 Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. *Nature* **277**, 294–297.
- Primmer, C. R., Saino, N., Møller, A. P. & Ellegren, H. 1998 Unravelling the process of microsatellite evolution through analysis of germ line mutations in barn swallows *Hirundo rustica*. *Mol. Biol. Evol.* **15**, 1047–1054.
- Queller, D. C., Strassmann, J. E. & Hughes, C. R. 1993 Microsatellites and kinship. *Trends Ecol. Evol.* **8**, 285–288.
- Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. 1998 Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494.
- Siegel, S. & Castellan Jr, N. J. 1989 *Nonparametric statistics*. New York: McGraw-Hill.
- Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M. & Clutton-Brock, T. H. 2000 Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proc. R. Soc. Lond. B* **267**, 1657–1662.
- Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*. New York: Freeman.
- Thursz, M. R., Thomas, H. C., Greenwood, B. M. & Hill, A. V. S. 1997 Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nature Genet.* **17**, 11–12.
- Van Noordwijk, A. J. & Scharloo, W. 1981 Inbreeding in an island population of the great tit. *Evolution* **35**, 674–688.
- Westerdahl, H., Bensch, S., Hansson, B., Hasselquist, D. & Von Schantz, T. 1997 Sex ratio variation among broods of great reed warblers *Acrocephalus arundinaceus*. *Mol. Ecol.* **6**, 543–548.
- Westerdahl, H., Bensch, S., Hansson, B., Hasselquist, D. & Von Schantz, T. 2000 Brood sex ratios, female harem status and resources for nestling provisioning in the great reed warbler. *Behav. Ecol. Sociobiol.* **47**, 312–318.
- Wheelwright, N. T. & Mauck, R. A. 1998 Philopatry, natal dispersal, and inbreeding avoidance in an island population of savannah sparrows. *Ecology* **79**, 755–767.