Absence of heterozygote advantage in a population of fallow deer *Dama dama* L.

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Abstract: Effects of inbreeding on individual fitness are of long-standing interest in evolutionary biology and conservation studies. Since estimation of the inbreeding coefficient of a given individual is often impossible in wild populations, some authors have attempted to demonstrate inbreeding depression in such populations by correlating the multi-locus marker heterozygosity of individuals with a trait that is assumed to be associated with fitness. A certain consensus exists, suggesting that associations between multi-locus heterozygosity and fitness-related components are relatively common. Nevertheless, negative results often go unreported and could thus lead to a bias in our overall understanding of inbreeding effects in wild populations. Here we investigate the effect of heterozygosity level in a population of fallow deer *Dama dama*. We found an absence of correlations between heterozygosity at a set of 20 expected neutral microsatellite markers and two fitness-related components (birth weight and winter survival of fawns). We discuss these results in relation to the characteristics of the studied population.

Keywords: heterozygote advantage, fitness, *Dama dama*, microsatellite

INTRODUCTION

Evolutionary and conservation biologists have a long-standing interest in the consequences of inbreeding (e.g. FRANKHAM 1998). Within a population a number of factors may lead to a decrease in genetic variability. These include matings between close relatives or between more distant coancestors, as well as genetic drift, which occurs over a longer time scale when populations are relatively small and isolated. This decrease in genetic variability is expected to have an important impact on the viability of fragmented populations (FRANKHAM 1995, SACCHERI et al. 1998,
because it may result in a reduction of the mean phenotypic value of traits associated with fitness (e.g. survival, fecundity, parasite load) or inbreeding depression (e.g. CHARLESWORTH & CHARLESWORTH 1987, LYNCH & WALSH 1998, CASSINELLO et al. 2001 in captive and domestic animals; COLTMAN et al. 1998, KELLER 1998, SLATE et al. 2000 in wild populations).

Estimating the inbreeding coefficient of a given individual is often impossible in wild populations because pedigrees are not available. In order to demonstrate inbreeding depression in such populations, we can exploit the fact that inbreeding reduces heterozygosity by correlating the multi-locus marker heterozygosity of individuals with a trait that is assumed to be associated with fitness (ALLENDORF & LEARY 1986). Such attempts revealed that associations between marker heterozygosity and fitness components are common when highly polymorphic neutral microsatellite markers are used and inbreeding depression is regarded as the most likely explanation for the relationship (e.g. COLTMAN et al. 1998 in the harbour seal Phoca vitulina, ROWE et al. 1999 in the natterjack toad Bufo calamita, BIERNE et al. 2000 in the oyster Ostrea edulis, MARSHALL & SPALTON 2000 in the Arabian oryx Oryx leucoryx, COULSON et al. 1998 and SLATE et al. 2000 in the red deer Cervus elaphus). Nevertheless, it is difficult to generalize about fitness advantages of heterozygosity in the wild because heterozygosity-fitness correlations are often very weak and their biological relevance has been questioned (e.g. DAVID 1998, AMOS et al. 2001) and because negative results are under-reported (BOOTH et al. 1990, WHITLOCK 1993, BRITTON 1996, DAVID 1998, HANSSON & WESTERBERG 2002). Moreover, alternative processes, which do not require inbreeding, can be responsible for such a correlation (DAVID 1998, HANSSON & WESTERBERG 2002, SLATE et al. 2004) and microsatellite-derived metrics used to infer relative inbreeding among individuals may be only weakly correlated with inbreeding coefficients (BALLOUX et al. 2004, PEMBERTON 2004, SLATE et al. 2004).

Here we investigate the effect of inbreeding in a population of fallow deer Dama dama. During the Pleistocene, fallow deer ranged over much of Europe, while during the later stages of the epoch they became restricted to more southern regions. Reintroductions by Romans, Phoenicians and Normans within historical time allowed the spread of fallow deer populations. The domestication process and the selection for tameness and coat colour (RANDI & APOLLONIO 1988) have led to an overall low genetic diversity among populations of fallow deer (PEMBERTON & SMITH 1985, HARTL et al. 1986, RANDI & APOLLONIO 1988 for allozyme loci; POETSCH et al. 2001, SAY et al. 2003 for microsatellite loci). Nevertheless, reduced genetic diversity of the species does not appear to have led to a decrease in survival or fecundity. In fact, most females reach puberty as yearlings and reproductive rates amongst adult females are high (PUTMAN 2003).

In order to clarify consequences of inbreeding on fitness in fallow deer we examined the relationship between two fitness-related components (birth weight and winter survival of fawns) and the marker heterozygosity at 20 microsatellite loci in an enclosed population living in the extensive Phoenix Park in Dublin (Ireland).
MATERIAL AND METHODS

Study area and population
The study population is enclosed in the Phoenix Park, North West of Dublin City, Ireland. It covers 709 ha, of which 569 ha are available to the deer. About 80% of the park area is covered by grassland, 20% by woodland. The population was founded when the park was enclosed in 1662. There has been a number of documented introductions since then, the most recent of which took place in 1906 (HAREL 1898, HAYDEN et al. 1992). Population size reached 700 individuals in 1924, but the deer were almost entirely culled during the Second World War. The current population comes from the less than 40 individuals that survived the war. During our study, population size exceeded 600 individuals and was characterised by a slightly female-biased sex ratio. From 1971 until present, fawns have been tagged annually. Approximately 70% of the fawns are successfully caught and tagged each year. During 1991 and 1992 the entire population was caught so that all individuals born prior to these years are tagged.

Measure of fitness components
One hundred and ten and 152 fawns born in 2001 and 2002, respectively, were caught by hand or with the help of a net shortly after birth between June and July each year. Ear tissue was sampled and preserved in 100% ethanol for the genetic analysis. Fawns were weighed to the nearest one-tenth of a kilogram using a Salter Electro Samson balance (Salter Weight-Tronix Ltd., West Bromwich, UK), tagged with coloured and numbered ear tags and released. In order to assess the survival of fawns through their first winter, their presence or absence in the population was noted in April of the year following birth (i.e. April 2002 for the 2001 cohort and April 2003 for the 2002 cohort) using Kawa telescopes (27× and 40× lens magnification). Fawns not observed during the month of April or subsequently in the population were presumed dead.

Most fallow deer mortalities are within the first year of life (CHAPMAN & CHAPMAN 1975, McELLIGOTT & HAYDEN 2000). Birth weight has a strong influence on both the future survival and the lifetime reproductive success of an individual in deer species (CLUTTON-BROCK et al. 1988, GAILLARD et al. 1997, KRUIK et al. 1999). We consequently used birth weight and first winter survival as estimators of the fitness of the fawns.

Measure of individual heterozygosity
Two main indicators of inbreeding are classically used: the multilocus individual heterozygosity ($H$), defined as the number of heterozygous loci divided by the total number of loci analysed, and the $d^2$ index proposed by COULSON et al. (1998). The latter ($d^2$) is a genotypic index that depends on the mutational dynamics of the marker locus, rather than its heterozygosity. It is specific for microsatellite loci and can be defined as the square of the difference in length of the two microsatellite alleles at a marker locus in an individual. In this study we calculated both $H$ and $d^2$ for each fawn. However, TSITRONE et al. (2001) previously showed, using simulations based on the two prevalent models of the mutation process and a variety of population
scenarios, that inbreeding coefficients should be more highly correlated with marker $H$ than with marker $d^2$ (see also, for example, Hedrick et al. 2001, Goudet & Keller 2002, Slate & Pemberton 2002, Markert et al. 2004). We consequently only report the analysis of marker H as an indicator of inbreeding in our study. It is however worth noting that our results for the $d^2$ index were analogous with those of the marker $H$. The genotypes of the 262 fawns in this study were previously determined at 20 microsatellite loci (see Say et al. 2003 for methods).

Statistical analyses

Statistical analyses were carried out separately on the two cohorts. Multi-locus and single-locus genetic diversity (number of alleles, heterozygosity observed and expected) as well as tests for deviation from the Hardy-Weinberg equilibrium were implemented using GENETIX 4.4 (Belkhir et al. 1996–2000).

We studied the relationship between the multi-locus individual heterozygosity ($H$) – arcsin square-root transformed for normalisation – and the birth weight of fawns using an ANCOVA with the sex of fawns as co-factors. Relationship between offspring survival (each fawn was categorised as 0 if dead or 1 if alive in April of the year following birth) and $H$ was analysed using generalised linear models (GLM) with a binomial error. Those analyses were performed using Splus (StatSci Mathsoft) software.

RESULTS

The mean number of alleles per locus was 3.4 (range: 2–7) for the 2001 cohort and 3.8 (range: 2–8) for the 2002 cohort (Table 1). Genotype frequencies at all loci except SR-CRSP1 did not differ significantly from Hardy-Weinberg expectations. The frequency of heterozygotes observed at each locus varied from 0.15 (locus BM4505) to 0.81 (locus MAF70) for the 2001 cohort and from 0.21 (locus BM4505) to 0.89 (locus MAF70) for the 2002 cohort. Among fawns, the multi-locus individual heterozygosity ($H$) values ranged from 0.10 to 0.68 for the 2001 cohort (mean±S.E.: 0.42±0.01) and from 0.12 to 0.77 for the 2002 cohort (0.45±0.02).

Birth weights of 71 and 73 fawns in 2001 and 2002 respectively, were recorded in the first two days of life (representing more than 60% of the total number of fawns born each year) and were used in the subsequent analyses. Males tended to be heavier at birth than females for the 2001 cohort ($F_{1,67}=2.95$, $P=0.09$) and were significantly heavier for the 2002 cohort ($F_{1,69}=7.63$, $P=0.01$). Nevertheless, the relationship between multi-locus individual heterozygosity $H$ and birth weight was not affected by the sex of the fawns ($F_{1,67}=3.26$, $P=0.08$ for 2001 and $F_{1,69}=0.12$, $P=0.73$ for 2002). We found no significant relationship between multi-locus individual heterozygosity $H$ and birth weight of fawns born respectively in 2001 ($F_{1,67}=0.001$, $P=0.98$) and 2002 ($F_{1,69}=1.22$, $P=0.27$) in our population (Fig. 1).

In total, 24 (22%) and 59 (39%) tagged fawns died during the winters of 2001 and 2002, respectively. Of these, respectively, 22 and 34 were weighed during their first two days of life and were used thereafter in the analysis. Winter survival of fawns was not significantly different in male and female fawns of the 2001 cohort ($\chi^2=0.17$, $P=0.68$). The same results were observed for the 2002 cohort ($\chi^2=0.19$, $P=0.66$). The
fawns that survived winter did not have significantly higher multi-locus individual heterozygosity values than those that died ($\chi^2=0.85$, $P=0.36$ for the 2001 cohort and $\chi^2=0.19$, $P=0.66$ for the 2002 cohort).

**DISCUSSION**

Here we report an absence of correlation between heterozygosity at a set of 20 expected neutral microsatellite markers and two fitness-related components (birth weight and winter survival of fawns) in an enclosed population of fallow deer. This result contradicts previous reports suggesting that ungulate species are particularly sensitive to a reduction of heterozygosity: an apparent heterozygote advantage has been reported in the red deer (COULSON et al. 1998, SLATE et al. 2000), Arabian oryx (MARSHALL & SPALTON 2000) and Soay sheep *Ovis aries* (COLTMAN et al. 1999).

Apparent heterozygote advantage may result from effects of homozygosity at selected loci in linkage disequilibrium with microsatellites (local effect hypothesis).
and from effects of homozygosity at genome-wide distributed selected loci under identity disequilibria mainly generated by inbreeding (general effect hypothesis, HANSSON & WESTERBERG 2002). Both phenomena could have led to correlations between heterozygosity and fitness-related components in our study. The Phoenix Park population of fallow deer experienced a strong bottleneck followed by a rapid population expansion that may have led to significant levels of linkage disequilibrium. In addition, this population is enclosed and no introductions have taken place since 1906, thus restricting opportunities for mating and fostering inbreeding among relatives.

Several reasons may explain the absence of heterozygote advantage here; reasons that could reflect a true lack of inbreeding depression, a lack of sufficient statistical power or the fact that multilocus heterozygosity is a poor indicator of inbreeding coefficient. First, some authors pointed out that inbreeding avoidance mechanisms might reduce inbreeding and thus inbreeding depression in wild populations (PUSEY & WOLF 1996). Male copulation success in fallow deer is age-related and a given male is unlikely to be highly successful in more than one year (MCÉLLIGOTT & HAYDEN 2000), so the possibility of a father-daughter or mother-son mating might only arrive in one year of the female’s life. However, given the skew in male copulation success (SAY et al. 2003), there is also the additional possibility of brother-sister mating. Although this possibility could be reduced if females avoided mating with males from their own cohort, there is no evidence in this population to suggest that females actively avoid brother-sister matings (FARRELL 2002). Secondly, the bottleneck experienced by the Phoenix Park population of fallow deer may have been strong enough to purge the population of deleterious recessive mutations, which consequently may have reduced the sensitivity of the population to inbreeding depression. This process is, however, only efficient under restricted genetic and demographic circumstances (KELLER & WALLER 2002) that are infrequently found in captive or wild populations (e.g. BALLOU 1997).

Heterozygosity-fitness correlations are often very weak and SLATE & PEMBERTON (2002) stated that a sample of more than 1000 individuals genotyped for more than 10 loci might be required to reveal any apparent heterozygote advantage. Large

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Fig. 1: Relationship between the multi-locus individual heterozygosity (arcsin square-root transformed values of $H$) and the birth weight of male △ and female ● fawns.
sample size is particularly important in enclosed non-natural populations, where animals are relatively homogenous in terms of genetic diversity and, consequently, where a relationship between individual heterozygosity and fitness components is difficult to highlight (AMOS et al. 2001, TSITRONE et al. 2001). While in this study individuals were genotyped for 20 loci, we only analysed approximately 100 fallow deer in each year. Although this number may seem relatively low according to the recommendations of SLATE & PEMBERTON (2002), it actually represented more than 60% of the complete cohort.

According to MEAGHER et al. (1997) and CRNOKRAK & ROFF (1999) apparent heterozygote advantage should be easier to highlight for populations living in harsh environmental conditions. In Phoenix Park, however, despite the high rate of winter survival reported here, the population is maintained well below the carrying capacity of the environment. Food is thus not limiting. Furthermore, although fawns may be killed by domestic dogs, foxes and perhaps badgers during their first few weeks of life, no death during this study can be definitively attributed to predation. Incidence of disease or infection (including external and internal parasites and bovine tuberculosis) in this population is very low (John Moriarty, personal communication) and the principal causes of fawn mortality in Phoenix Park are road-traffic accidents. A death caused by a road-traffic accident is, however, a hazardous event and is not considered a consequence of natural selection. Therefore, winter survival of fawns may be a poor estimator of individual fitness in our population of fallow deer.

Similarly, in recent papers SLATE et al. (2004) and BALLOUX et al. (2004) questioned the use of heterozygosity across microsatellite loci as a measure of relative inbreeding among individuals, suggesting that it may be an inappropriate method of investigating inbreeding depression in many natural populations. Most significantly SLATE et al. (2004) showed that multi-locus heterozygosity is only weakly correlated with inbreeding coefficient and that multi-locus heterozygosity, but not inbreeding coefficient, failed to detect evidence of inbreeding depression for morphological traits in a population of New Zealand domestic sheep *Ovis aries*.

To conclude, although published works to date have suggested that associations between multi-locus heterozygosity and fitness-related components are relatively common (BRITTEN 1996), negative results are not infrequent (SAVOLAINEN & HE-DRICK 1995, CRNOKRAK & ROFF 1999). Due to the fact that they often go unreported (WHITLOCK 1993), the absolute number of these exceptions is difficult to evaluate and is probably greatly underestimated. It is therefore important to report negative results and great caution should be taken when choosing a particular method to estimate fitness and genome-wide heterozygosity (PEMBERTON 2004) to avoid biasing our overall understanding of inbreeding effects in wild populations and to provide a better understanding of the modalities that influence the expression of the heterozygote advantage.

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