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Estimation of the rate of inbreeding in a natural population of *Adalia bipunctata* (Coleoptera: Coccinellidae) using a phenotypic indicator

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Abstract. The rate of inbreeding is an important parameter in the population and evolutionary genetics of a species. Accurate measurement of the rate of close inbreeding can be gained through the use of DNA fingerprinting; however, this is both labour intensive and expensive. We here provide a more approximate measure of the rate of close inbreeding in *Adalia bipunctata*, utilising the unusual phenotypic effect of inbreeding, the production of eggs which develop but fail to hatch, which is seen in this species. Examination of the incidence of grey eggs in known outbred and inbred laboratory crosses and comparison to their incidence in wild-collected clutches produced an upper estimate of 2.7% of wild clutches being the product of close inbreeding. This result is discussed in the light of the ecology and evolutionary biology of *Adalia bipunctata*.

INTRODUCTION

Inbreeding rates are an important parameter in our understanding of the evolutionary biology of a species. In particular, they affect the pattern of gene flow and the degree of adaptation to the local environment, the evolution of social behaviour, and the tendency of a species to show heterosis. The presence of inbreeding in populations of insects has also been held as a reason for the spread of male-lethal symbionts (Lus, 1947a; Werren, 1987).

We here attempt to gain some insight into the rate of inbreeding in natural populations of the ladybird *A. bipunctata*. Past attempts to measure the rate of inbreeding in natural populations have adopted two different methodologies. First, the rate of inbreeding has been estimated by the examination of natural variation between individuals at polymorphic loci. Inbreeding tends to increase homozygosity, and comparison of the observed frequency of homozygotes with that expected under random mating (calculated using the Hardy-Weinburg formula) may represent a test of the level of inbreeding. However, such a method is generally poor at detecting low levels of inbreeding, and may be confounded by other factors which produce an excess of homozygotes, such as the Wahlund effect (Wahlund, 1928). Second, the rate of inbreeding has been assessed directly, through calculation of the average relatedness of mating pairs. Either the characterization of individuals from a large number of mating pairs for genetic markers such as allozymes which show a low level of variability, or the characterization of members of fewer pairs for a more variable genetic marker such as a minisatellite repeat, may allow the production of an estimate of the rate of inbreeding. Although this approach is feasible (e.g. Quezada-Diaz et al., 1992), it tends to be both time-consuming and costly.

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An alternative to the above is to examine the population for some phenotypic indicator of inbreeding. This approach is the one used here to examine *A. bipunctata* populations for evidence of inbreeding. Past studies on inbreeding in *A. bipunctata* indicate the species to have an abnormally high load of recessive lethal genes (Lus, 1947b; Majerus, 1994): inbreeding depression is severe. Laboratory crosses between siblings produce eggs which have a high rate of inviability. Inviable eggs may be classified into two kinds: grey ones, where a fully-developed grey segmented embryo is visible inside the chorion, and yellow ones where the embryo is not visible. We here quantify the rate of sibling-sibling inbreeding in a natural population by comparing the fate of eggs (in terms of hatching and colour) in naturally occurring clutches with the fate of eggs of known parentage, the product of either inbreeding or outbreeding in the laboratory. The study focuses upon the rate of production of grey eggs, because, in this species, the production of yellow eggs may be a manifestation not just of inbreeding, but also of the presence of a male-lethal symbiont (Lus, 1947a; Hurst et al., 1992).

MATERIAL AND METHODS

Measurement of the phenotypic effect of inbreeding

Individual larvae were collected from two closely situated sites within Cambridge: from lime trees (*Tilia* x *europaea*), and from nettles (*Urtica dioica*). Larvae from each site were reared separately to maturity, and adult females crossed randomly and repeatedly to males collected from the other site. Egg clutches were collected from each female, and the larvae reared to maturity to form families of siblings and half-siblings, the progeny sharing a mother, but only possibly sharing a father. Two females from each of these families were removed and fed on excess of pea aphid (*Acrythosiphum pisum*) for seven days. One of each pair of females was then outbred, being crossed with a male collected from Coton (three miles west of Cambridge); the other was inbred, being crossed with her brother (or half-brother). The breeding pairs were maintained on a diet of excess pea aphids under 24 hour light at laboratory temperatures (20–25°C). Clutches of eggs were collected from each pair, and egg-fate recorded in terms of the number which hatched, and the colour of those which failed to hatch. Eggs were scored as grey if following larval dispersal from the clutch they remained unhatched, with the shrivelled embryo clearly visible through the chorion. Only clutches containing more than 10 eggs were scored from each pair, and a maximum of five clutches was recorded to prevent bias in the data toward particular crosses.

Egg-fate in naturally occurring clutches

Hatching egg clutches containing more than 10 eggs were collected from *Salix alba* (willow), *Tilia* x *europaea* (lime) and *Prunus domestica* (plum) in the Cambridge area during May–June 1992. The hatched larvae were allowed to disperse from the natal clutch, and the fate of the eggs characterised, as above. Clutches from the other major host plant in the Cambridge area, *Urtica dioica* (nettle), were not used because, although available, egg clutches on nettle leaves are frequently damaged when removed to the laboratory. The leaves of nettle desiccate rapidly after detachment, causing the leaf to curl up. This can cause eggs to be crushed or distorted, and leads to artifactual decreases in egg hatch-rate.

RESULTS

Egg-fate, in terms of the number of grey eggs, was strongly affected by inbreeding. A total of 110 clutches from 27 outbred laboratory pairs were recorded. Around 8% of the eggs in these clutches were scored as grey, having failed to hatch following (apparently) full development (Fig. 1A). A total of 99 clutches from 21 inbred laboratory crosses were obtained. Approximately 26% of the eggs from these clutches were scored as grey (Fig. 1B). Inbreeding can thus be seen to be associated with a large increase in the frequency of grey eggs in clutches (Mann-Whitney test: $U = 2197$; $n1 = 110$, $n2 = 99$; $p < 0.0001$).

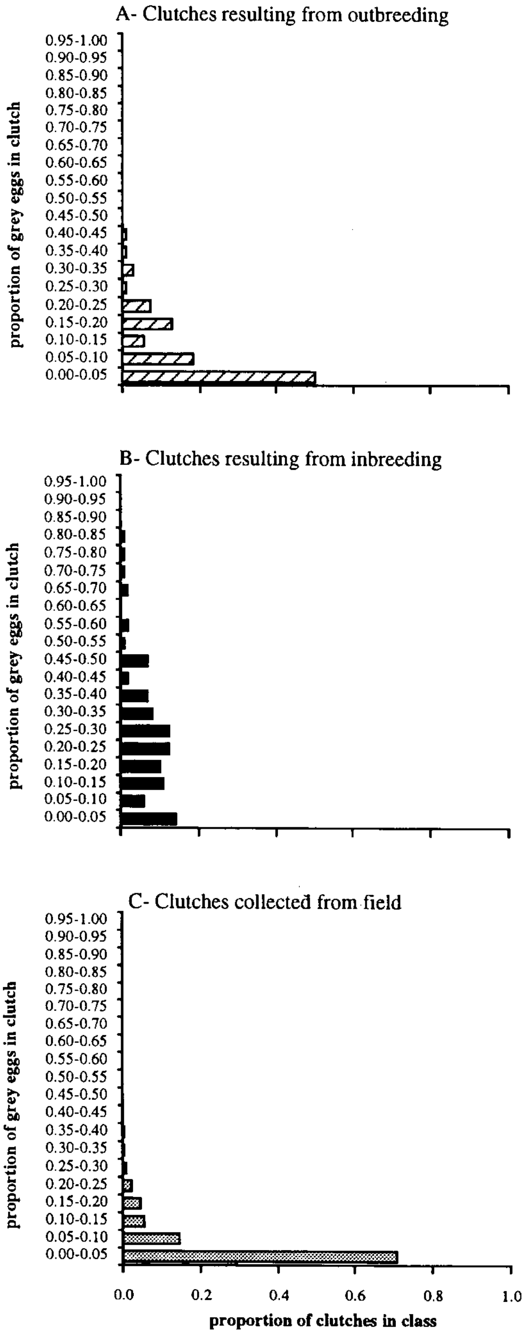

Fig. 1. The proportion of eggs failing to hatch following significant embryonic development (grey eggs): A – clutches that are the product of outbreeding in the laboratory; B – clutches that are the product of inbreeding in the laboratory; C – clutches collected from the field.

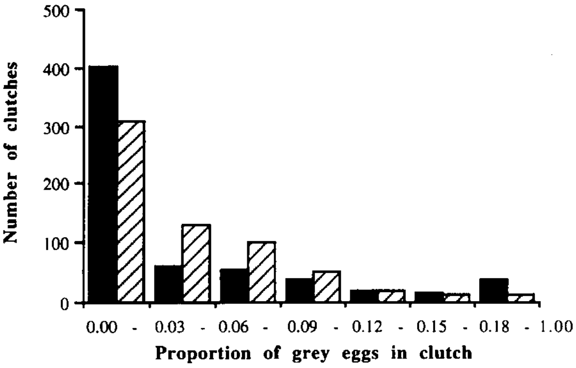

Fig. 2. The number of naturally occurring clutches with different proportions of eggs which fail to hatch following extensive development (filled bars), compared to the number of clutches expected to show this egg-fate if such eggs occur independently (hatched bars).

In comparison, a total of 636 clutches collected from the field were allowed to hatch. Only 4.1% of the eggs from these clutches were scored as grey (Fig. 1C). Grey eggs were found at a lower frequency in wild clutches than in both clutches produced by both outbred ($U = 26683$; $n1 = 110$, $n2 = 636$; $p < 0.0001$) and inbred ($U = 8423$; $n1 = 99$; $n2 = 636$; $p < 0.0001$) crosses in the laboratory. This indicates a general decrease in fertility as a consequence of laboratory rearing.

The distribution of occurrence of "grey eggs" in naturally occurring clutches did not appear to be random. More clutches contained a large number of eggs with this fate than expected by random chance (Fig. 2). The expected distribution of grey eggs in clutches, under the null hypothesis that fate of one egg is independent of the fate of others in the clutch, was calculated for each clutch size, and (by weighting each of these distributions by the number of times clutches of that size appeared in the sample) for the sample as a whole. The incidence of such eggs is non-random ($\chi^2 = 68.2$; 6 d.f.; $p < 0.0001$).

CONCLUSION

Inbreeding is associated with a change in egg-fate. In particular, the production of "grey eggs", eggs which fail to hatch following full embryonic development, is increased. The viability of eggs produced by laboratory-reared parents differs from eggs collected from the field: the eggs collected from the field showed a higher probability of hatching following development, than both laboratory-reared eggs known to be the product of outbreeding, and laboratory-reared eggs known to be the product of inbreeding. This is not surprising, given the differences in the environmental influences on laboratory and naturally reared ladybirds. It means that the rate of inbreeding cannot be estimated accurately by a formal comparison of the egg-fate from naturally occurring and laboratory produced clutches. However, the low frequency of "grey eggs" in clutches in the wild, compared to the massive rate of production of such eggs following inbreeding observed above, suggests that the rate of close inbreeding in the wild is very low.

The question is how low? This cannot be precisely assessed. However, insight may be gained from clutch-by-clutch analysis of egg-fate in naturally occurring clutches. Inbreeding would be expected to produce a degree of heterogeneity in the data. Clutches that were the product of inbreeding would be expected to have aberrantly high numbers of "grey eggs". As may be seen above, a greater number of naturally occurring clutches have a high proportion of "grey eggs" than would be expected by chance. If egg-fates within the data set are independent, then only 23 clutches would be expected to contain more than 15% "grey eggs". A total of 39 such clutches were observed. Sixteen of the 636 clutches collected, 2.7%, have lower egg viabilities than expected by chance.

How can we explain the presence of this small excess of clutches containing large numbers of these eggs? One explanation is that they are the product of inbreeding. However, increased numbers of "grey eggs" are not necessarily the product of inbreeding. First, it may be that some females, because of their poor condition, produce eggs with lowered viability. Observations of laboratory crosses, for instance, suggest that eggs produced shortly before the death of their mother have lower viability than eggs produced at other times (Hurst, pers. obsn.). Second, it may be that certain outbred matings produce eggs with low viabilities because of the genetic constitution of each parent. Genetic incompatibility of this kind is most obviously manifested in inbred clutches, where, due to common decent, both parents bear a single copy of a recessive lethal allele, such that a proportion of the progeny are homozygous for this allele, and therefore die. This mutational load is not, however, restricted to the progeny of inbred matings. The load is also borne when zygotes are formed from parents which, although being no more related than average, happen to bear the same lethal allele. In humans, where most individuals carry three to five lethal alleles, many genetic diseases are well characterised, and occur at significant rates, in a population which now mates fairly randomly with respect to relatedness. The same probably occurs in *A. bipunctata.*

Of the clutches that show high frequencies of developed but unhatched embryos, not all will be the product of inbreeding. If the number of suspect clutches is 2.7%, then it may be stated fairly safely that within the limits of sampling error, fewer than 2.7% of all clutches are the product of inbreeding. This is a maximum figure, and the rate of inbreeding may even be an order of magnitude lower than this estimate.

DISCUSSION

The low level of inbreeding in *A. bipunctata* found in this study accords with our knowledge of the ecology of this species. The species is one where dispersal occurs during the summer whenever a patch becomes devoid of aphids, thence to overwintering aggregations away from the summer breeding area, thence back to new host plants. The degree of mixis of adults in the period before oviposition is high, and this is consistent with a low rate of inbreeding.

The rarity of inbreeding leads us to several obvious conclusions. First, if *A. bipunctata* is not strongly inbred, we should not expect to see a high degree of heterosis in *A. bipunctata.* Second, the theory that the male-killing behaviour of inherited microorganisms has evolved because it reduces inbreeding avoidance is likely to be untrue for the male-killer in *A. bipunctata.* Other explanations need to be sought, and it is most likely that it is the presence of sibling egg cannibalism in this species that is most important in the evolution of this behaviour (Hurst et al., 1992; Hurst, 1993; Hurst & Majerus, 1993). Male-killing is favoured because the dead male is eaten by its sister (who also bears the bacterium), which therefore has an increased probability of survival.

What caveats should be placed upon the above results and conclusions? It should be noted that the above experiments and observations investigate the rate of inbreeding in the first summer generation of this species. This is the generation most likely to be outbred, due to the mixis created by dispersal to and from overwintering sites, usually human dwellings separate from the breeding sites. In cases where a second generation is produced in a year, the adults breeding late in the year may have undergone a lower level of mixis: *A. bipunctata* individuals will remain on their natal patch so long as aphids are abundant. This is evidenced by the heterogeneous distribution of adults of rare colour morphs over short distances late in the season. More than one adult of the unifactorial recessive form *duodecempustulata*, which occurs at an overall frequency of under half of one per cent, have been found on host patches supporting relatively few adult individuals (Majerus, pers. comm.). In this situation, the frequency of inbreeding may increase. The production of a second generation within a year is observed, but is not common in the U.K. (Majerus & Kearns, 1989). The proportionate contribution of this generation to the overwintering population is variable between years, but is on average low. The assessment of inbreeding frequency in Cambridge as being rare remains valid. However, two, or even three, generations a year are commonly observed in continental populations with warmer climates (Lus, 1947b). Here, if second and third generations mate primarily within demes, inbreeding will be more frequent.

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