

Disruptive selection and the genetic basis of bill size polymorphism in the African finch *Pyrenestes*

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MECHANISMS producing and maintaining discrete polymorphisms have long fascinated evolutionary biologists^{1,2}. Despite the ubiquity of non-sex-limited polymorphisms in vertebrates, the evolutionary factors maintaining them are well understood in only a few instances³. The African finch *Pyrenestes* is unique among birds in exhibiting a non-sex-determined polymorphism in bill size^{4,5}. Morphs breed randomly with respect to bill size and differ in diet and feeding performance on soft and hard seeds^{4,6}. I present here: (1) new data showing that the polymorphism appears to result from a single genetic factor; (2) support from long-term field studies for earlier suggestions that disruptive selection is acting on bill size; and (3) data revealing the presence of a possible third, much larger morph. Results suggest that the polymorphism may have arisen through single mutations, where morphs occupy distinct adaptive peaks through differences in feeding performance on seeds differing in hardness.

I conducted field work for three years between 1983 and 1990 on the black-bellied seedcracker (*P. o. ostrinus*) in Cameroon. Over 2,700 individuals were netted, banded and measured⁴. Evidence of natural selection on six morphological characters was examined over a 7-year period (Fig. 1). Because annual variation in rainfall and seed production was low^{6,7}, I assume the direction and intensity of selection varied little across years. I estimated selection over the entire study, rather than in each

year or across a single season, to maximize sample size. Previous research suggested that selection primarily takes place during the major dry season (February–May), during which food is least abundant and inter- and intraspecific competition is most intense⁷. Analysis centred on survival of juveniles because selection tends to be most intense on this age class⁸. Juveniles of either sex measured and released between 1983–1986 were compared with those individuals recaptured during an 8-month period in 1989 and 1990. I estimated natural selection on each of six morphological characters using the cubic spline technique⁹, which is a nonparametric technique that provides a quantitative prediction of survival probabilities across a range of differing character sizes, allowing an estimate of fitness for a particular character value, and a fitness surface for the character in the population. I found evidence of disruptive selection occurring on four of the six characters: lower mandible width, lower mandible length, upper mandible depth and tarsus length (Fig. 1). In each case there is evidence of a bimodal fitness surface with two peaks and a valley between them. No selection was detected on wing or upper mandible length.

Disruptive selection is probably related to seed quality. Bill size, particularly lower mandible width, is the most important character in predicting the time taken to crack seeds⁴ and has been shown to be the primary target of selection⁸. Morphs feed primarily on seeds of two species of sedge; these seeds are similar in size but differ dramatically in hardness. Large morphs feed more efficiently on the hard-seeded sedge (*Scleria verrucosa*, mean hardness = 153 Newtons, s.e. ± 2.0); small morphs feed more efficiently on the soft seeded sedge (*S. goossensii*, mean hardness = 13 N, s.e. ± 2.0)⁶. Despite evidence of disruptive selection on lower mandible length and tarsus, neither of these characters shows a bimodal distribution, in contrast to the other two characters on which selection occurred. This may suggest that components of fitness other than juvenile to adult survival may be acting on these characters.

In addition, during 1985 and 1989, 97 finches were exported from my main study site in south-central Cameroon (03°45' N,

FIG. 1 a, Fitness surfaces of the four characters showing evidence of natural selection calculated using the cubic spline technique. Splines were estimated for each character (solid lines), with confidence limits for each spline (dashed lines) generated by bootstrapping in which the original data set was resampled 100 times⁹. Shaded histograms (b) show distributions of juveniles that did not survive, black histograms those that survived. c, Distribution of characters in the adult population. Juveniles that were not captured in 1989–90 were assigned an absolute fitness of 0, and those recaptured, a value of 1. Only juveniles that were 2–3 months old and had already reached adult size⁵ were used in the analysis. Sampling adjacent regions to the study site revealed no evidence of differential dispersal of particular phenotypes. Because splines are not designed to handle characters with major gaps in their distributions (D. Schluter, personal communication), splines were calculated for each morph separately for lower mandible width. Additionally, because the performance of the bootstrap to estimate confidence limits has not yet been tested through simulation, bootstrap results should be interpreted cautiously⁹.

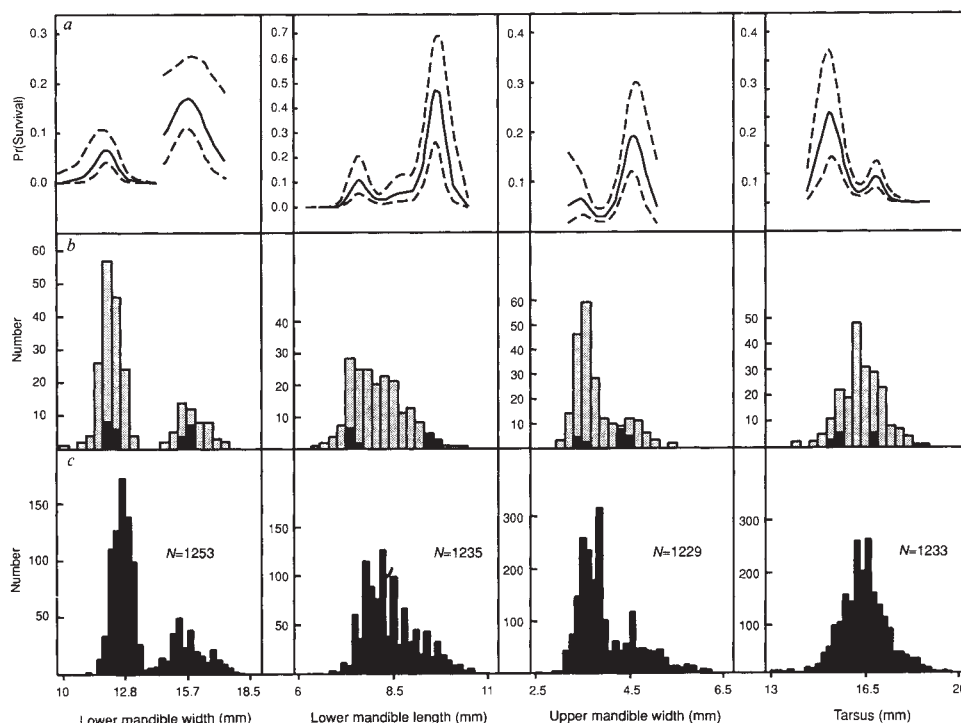
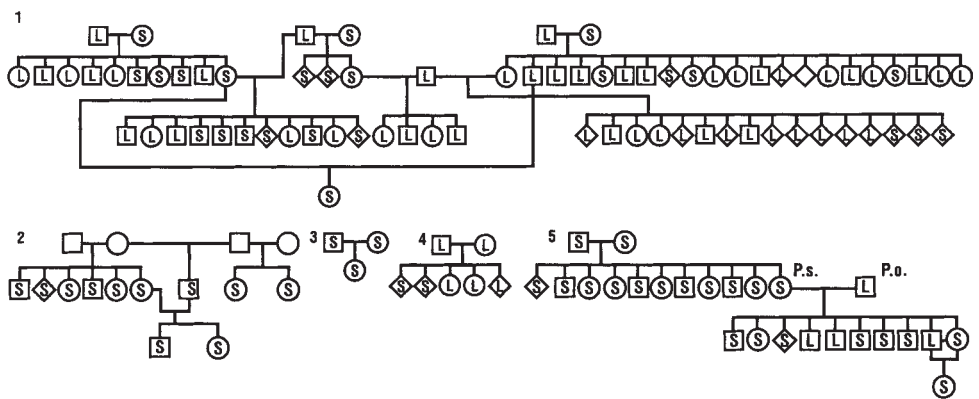


FIG. 2 Pedigrees showing matings between small (S)- and large (L)-billed morphs (lower mandible width <14 mm and >14 mm, respectively). Squares and circles represent adult males and females, respectively, triangles are juveniles less than 6 months old. Bill size was determined on the basis of lower mandible width which reaches adult size at four months of age⁵. Individuals in which bill size was not measured or in cases of juveniles which died before bill size could be determined are blank. Pedigrees 1 and 4 represent matings involving the race *P. o. ostrinus* (P.o.), matings 2 and 3 the race *P. o. sanguineus* (P.s.) and within pedigree 5 a cross between *P. o. sanguineus* and *P. o. ostrinus*. Heritabilities and repeatabilities for 6 morphological characters from 11 families and 69 offspring were calculated from regressions of offspring on mid-parents after data was \log_e transformed¹⁷. Only juveniles that had reached adult size were used in the analysis. Captive bred (and wild) nestlings reach near-adult size while being fed by their parents and do not crack seeds until after fledging. Thus, there is no opportunity for food hardness to affect morphology during early growth as occurs among morphs of some fishes¹⁸. All heritabilities except for tarsus



were significantly different from zero ($P < 0.01$). Heritabilities (\pm s.e.) and repeatabilities (%) for each character and generalized size and shape characters (principal component 1 and 2), are: wing length, 0.49 ± 0.17 , 29; lower mandible width, 1.13 ± 0.16 , 92; bill depth, 0.67 ± 0.14 , 88; upper mandible length, 1.25 ± 0.19 , 80; lower mandible length, 1.12 ± 0.27 , 82; PC1, 0.58 ± 0.10 ; PC2, 0.35 ± 0.11 . The number of other loci which may secondarily be involved in modifying the expression of the main locus is currently unclear.

12°15' E) to a breeding facility at the Riverbanks Zoological Park in Columbia, South Carolina. Pairs were housed under identical environmental conditions and fed a commercial finch diet¹⁰. Pedigrees (Fig. 2) show that all crosses produced offspring with either small or large bills, but not individuals with intermediate sized bills. Heterotypic pairs that produced mixed broods did so in a ratio not significantly different from 1:1 (21 small: 30 large, $\chi^2 = 0.64$, $P > 0.4$). Homotypic large pairs produced offspring in nearly a 3:1 ratio (16 l: 5 s), whereas all small homotypic pairs produced offspring with bill sizes like those of their parents. The distribution of lower mandible width in F_1 s produced from heterotypic pairs producing mixed broods is shown in Fig. 3. These results are consistent with the polymorphism being produced by a single autosomal di-allelic locus with complete dominance for large-bill.

Although *Pyrenestes* exhibits distinct bill morphs within single interbreeding populations with no apparent geographic segrega-

tion, an analysis of museum specimens revealed the occurrence of a third extremely large-billed form, occurring in ecotonal regions between forest and savanna⁵. In northern Cameroon, I found this megabilled form coexisting with small and large morphs (Fig. 4). The megabilled form fed primarily on a species of sedge (*S. racemosa*) with extremely hard seeds (mean hardness = 299 N, s.e. ± 11.9). A single breeding pair was found consisting of a small-billed male and a megabilled female, which is remarkable considering that megabilled forms are 31% heavier and have a bill over 47% larger¹¹.

In *Pyrenestes*, the precise role that disruptive selection plays in maintaining the polymorphism in an ecologically dynamic

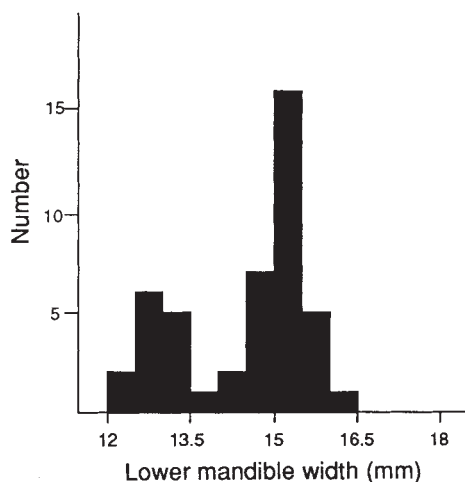


FIG. 3 Distribution of bill size in F_1 s produced from the heterotypic pairs producing mixed broods (Fig. 2). Lower mandible widths for the four pairs, sires and dams, respectively, are: 15.9 mm, 12.1 mm; 16.2, 12.4; 14.9, 12.7; 15.2, 12.6.

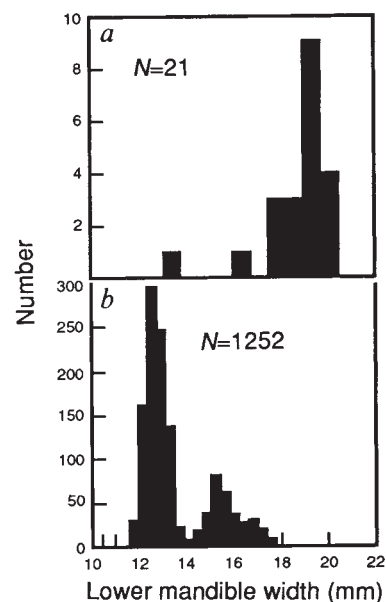


FIG. 4 a, Distribution of lower mandible width of adult males and females captured at Tibati (06°28' N, 12°38' E), in central Cameroon. Histogram shows the presence of small-, large- and megabilled individuals. b, The distribution of lower mandible width of adults of both sexes from the main study area in south central Cameroon is shown for comparison.

environment requires further research. But the apparent simple genetic basis of the polymorphism suggests that single mutations may have allowed populations to cross adaptive valleys^{12,13} in which larger morphs occupy separate adaptive peaks through differences in feeding performance on seeds of differing hardness. This hypothesis is supported by the most closely related species, *Spermophaga haematina*, exhibiting a bill similar in size and shape to the small morph of *P. ostrinus*, suggesting the ancestor of *Pyrenestes* also had a small bill⁶. Data further suggest that disruptive selection is important in producing and maintaining discrete polymorphisms² and that alternative adaptations may be maintained by polymodal selection¹⁴. Results also lend empirical evidence counter to the fisherian view that adaptations result primarily from allelic substitutions of slight effect at many loci^{15,16}, and supports recent views that major genes may be an important source of new adaptations¹². □

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Ecology of transgenic oilseed rape in natural habitats

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CONCERNS about genetically engineered crop plants centre on three conjectural risks: that transgenic crop plants will become weeds of agriculture or invasive of natural habitats; that their engineered genes will be transferred by pollen to wild relatives whose hybrid offspring will then become more weedy or more invasive; or that the engineered plants will be a direct hazard to humans, domestic animals or beneficial wild organisms (toxic or allergenic, for example). Here we describe an experimental protocol for assessing the invasiveness of plants. The object is to determine whether genetic engineering for herbicide tolerance affects the likelihood of oilseed rape becoming invasive of natural habitats. By estimating the demographic parameters of transgenic and conventional oilseed rape growing in a variety of habitats and under a range of climatic conditions, we obtain a direct comparison of the ecological performance of three different genetic lines (control, kanamycin-tolerant transgenics and herbicide-tolerant transgenic lines). Despite substantial variation in seed survival,

plant growth and seed production between sites and across experimental treatments, there was no indication that genetic engineering for kanamycin tolerance or herbicide tolerance increased the invasive potential of oilseed rape. In those cases in which there were significant differences (such as seed survival on burial), transgenic lines were less invasive and less persistent than their conventional counterparts.

We used genetically engineered plants grown in natural habitats to compare the demography of transgenic and conventional lines of plants in a range of habitats throughout Great Britain in order to find out how ecological performance is affected by genetic engineering. To quantify the effect on the invasive potential, we estimated the finite rate of increase (λ) of different genetic lines under a variety of experimental conditions. Values of $\lambda > 1$ (the invasion criterion) predict that the plant will increase in abundance under the given set of environmental conditions; values of $\lambda < 1$ predict that the plants will decline to extinction^{1,2}. The model system was the crop plant *Brassica napus* subsp. *oleifera* (oilseed rape variety Westar), engineered to express tolerance of the antibiotic kanamycin and tolerance of the herbicide glufosinate (marketed as Basta in Europe and as Challenge in the UK). Kanamycin is used as a selectable marker in the transformation process and is not expected to confer any advantage in the field. Glufosinate herbicide works by interfering with the plant's ammonium metabolism and the expression of a glufosinate-tolerant phenotype allows oilseed rape to be sprayed with herbicide, killing weeds without harming the crop. As neither antibiotics nor herbicides were applied in the experiments, we predicted that the transgenic lines would not outperform the conventional plants. It was not clear, however, whether the expression of a transgenic phenotype would impose a measurable cost in terms of reduced ecological performance by the transgenic lines (the 'genetic baggage' hypothesis^{3,4}).

Oilseed rape is an annual or short-lived monocarpic perennial with a simple life cycle. Seeds germinate in autumn or in the following spring, flowering and seed ripening occur during summer and seed is shed in early autumn. The experiments involved spring sowing (Table 1), so the finite rate of increase can be written as

$$\lambda_1 = (1 - d_1 - g) + g(1 - d_2)\bar{F} \quad (1)$$

where d_1 is the proportion of seeds that die in one full year, g is the proportion of seeds germinating in the first spring, d_2 is the proportion of seeds that die over winter, and \bar{F} is the mean number of seeds produced per seed that germinates^{2,5}. The first term on the right-hand side refers to the carry-over of seed from one year to the next, and the second term calculates the number of seeds produced by those plants that germinate and grow in competition with native vegetation. Separate experiments were carried out to evaluate each component: a seed burial experiment gave estimates of d_1 (and hence dormancy), whereas a seed sowing experiment provided estimates of germination, plant survival and fecundity. The experiments were carried out over three years in 12 different habitats at three sites within Great Britain (Sutherland, Cornwall and Berkshire); each experiment was replicated four times (details given in Table 1).

Seedling densities, adult plant densities and mean seed production per plant all varied significantly between years and between habitats within years (Table 1). There was no significant effect of genetic line on any of the demographic parameters, nor were there any interactions between sites, years and genetic line. Some of our experimental treatments had major effects on plant demography (cultivation to reduce interspecific competition, fencing to exclude vertebrate herbivores, chemical exclusion of molluscs), whereas others had none (exclusion of insect herbivores or fungal pathogens); these results will be presented elsewhere.

Seeds buried in each of the habitats at two depths (2 cm and 15 cm) were retrieved after 12 and 24 months and their fate