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Experimentally induced life-history evolution in a natural population

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LIFE-HISTORY theory predicts that reduced adult survival will select for earlier maturation and increased reproductive effort; conversely, reduced juvenile survival will select the opposite[1–5]. This is supported by laboratory studies[6–10] and comparative data from natural populations[11–15]. Laboratory studies may support a theory, but cannot assess its importance in natural populations, and comparative studies reveal correlations, not causation[16]. Long-term perturbation experiments on natural populations resolve both problems. Here we report the findings of a long-term study of guppies (*Poecilia reticulata*), in which the predictions of life-history theory are supported. Life-history differences among populations of guppies are closely associated with predator species with which guppies live[13,17–21]. The predators apparently alter age-specific survival because they are size-specific in their choice of prey[21–23]. *Crenicichla alta* (a cichlid), the main predator at one class of localities, preys predominantly on large, sexually mature size classes of guppies[22–24]. *Rivulus hartii* (a killifish), the main predator at another class of localities, preys predominantly on small, immature size classes. Guppies from localities with *Crenicichla* mature at an earlier age, have higher reproductive effort, and have more and smaller offspring per brood than those from localities with just *Rivulus*. These differences are heritable, and correspond with theoretical predictions[17–19]. To prove that predation caused this pattern, we perturbed a natural population of guppies by changing predation against adults to predation against juveniles. This resulted in significant life-history evolution in the predicted direction after 11 years, or 30–60 generations.

In 1976, guppies were transplanted from a site on the Aripo River (Trinidad) with *C. alta* (control site) to a tributary of the Aripo which previously contained *R. hartii* but no guppies (introduction site)[24]. This manipulation released guppies from selective predation on adults and exposed them to selective predation on juveniles. This treatment should favour guppies with delayed maturity and decreased reproductive effort, compared to the control site. The founding population size was 200 adults[24], consisting of equal proportions of males and non-virgin females. As guppies have sperm storage and multiple mating, the effective founding population size was almost certainly greater than 200; founder effects are therefore unlikely. In all

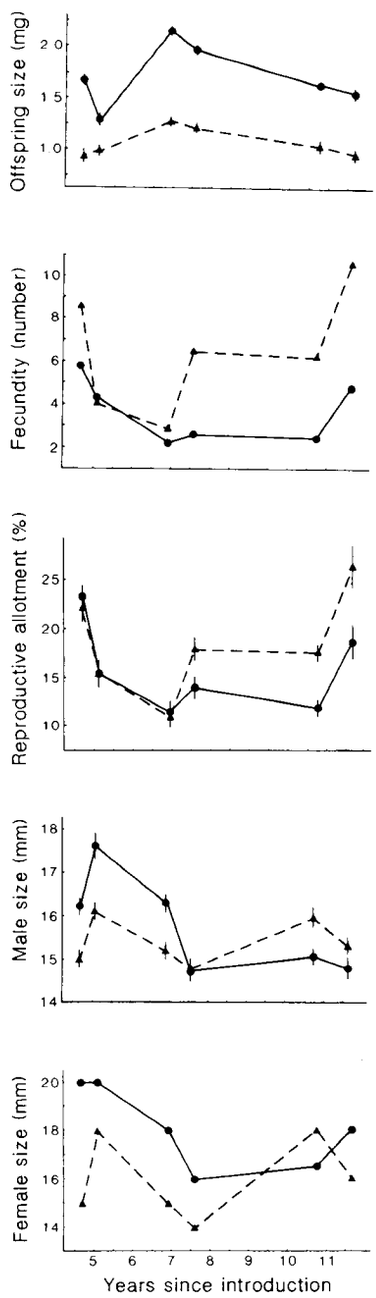

FIG. 1 Life-history phenotypes of guppies from the introduction and control sites. These data are based on wild-caught, field preserved fish. Offspring Size, mean dry weight of developing embryos, corrected for their stage of development and female size. Fecundity, expected number of offspring for a 30 mg (somatic dry weight) female. Reproductive Allotment, per cent of total dry weight that consists of developing embryos, and is corrected for the stage of development of the embryos. Male Size, average size of sexually mature males. Female Size, minimum mm size class in which the majority of females were carrying developing embryos. ●, Introduction site. ▲, Downstream control. All values are least-square means and one standard error from analyses of variance. All statistical comparisons were made within a collection and were executed with the SAS GLM procedure[31].

TABLE 1 Aripo tributary introduction

Life history trait*	Reznick and Bryga (1987)[21] Control (*Crenicichla*)	Reznick and Bryga (1987)[21] Introduction (*Rivulus*)	This study Control (*Crenicichla*)	This study Introduction (*Rivulus*)	Reznick (1982)[17] *Crenicichla*[17]	Reznick (1982)[17] *Rivulus*[17]
Male age at maturity (days)	60.6 (1.8)	72.7 (1.8)†	48.5 (1.2)	58.2 (1.4)†	51.8 (1.1)	58.8 (1.0)†
Male size at maturity (mg-wet)	56.0 (1.4)	62.4 (1.5)†	67.5 (1.2)	76.1 (1.9)†	87.7 (2.8)	99.7 (2.5)†
Female age at first parturition (days)	94.1 (1.8)	95.5 (1.8) (NS)	85.7 (2.2)	92.3 (2.6)‡	71.5 (2.0)	81.9 (1.9)†
Female size at first parturition (mg-wet)	116.5 (3.7)	118.9 (3.7) (NS)	161.5 (6.4)	185.6 (7.5)†	218.0 (8.4)	270.0 (8.2)†
Brood size, litter 1	2.5 (0.2)	3.0 (0.2) (NS)	4.5 (0.4)	3.3 (0.4)‡	5.2 (0.4)	3.2 (0.5)†
Brood size, litter 2	6.3 (0.3)	7.0 (0.3)§	8.1 (0.6)	7.5 (0.7) (NS)	10.9 (0.6)	10.2 (0.8) (NS)
Brood size, litter 3‖	—	—	11.4 (0.8)	11.5 (0.9) (NS)	16.1 (0.9)	16.0 (1.1) (NS)
Offspring size (mg-dry), litter 1	0.91 (0.02)	0.87 (0.02) (NS)	0.87 (0.02)	0.95 (0.02)§	0.84 (0.02)	0.99 (0.03)†
Offspring size, litter 2	0.93 (0.02)	0.86 (0.02)‡	0.90 (0.03)	1.02 (0.04)‡	0.95 (0.02)	1.05 (0.03)‡
Offspring size, litter 3‖	—	—	1.10 (0.03)	1.17 (0.04) (NS)	1.03 (0.03)	1.17 (0.04)†
Interbrood interval (days)	24.9 (0.4)	24.89 (0.4) (NS)	24.5 (0.3)	25.2 (0.3) (NS)	22.8 (0.3)	25.0 (0.03)†
Reproductive effort (%)#	4.0 (0.1)‡	3.9 (0.1) (NS)	22.0 (1.8)	18.5 (2.1) (NS)	25.1 (1.6)	19.2 (1.5)‡

Values are means (s.e.), and represent data from refs 17 and 21, or this study.
NS, not significant.
* Differences in mean values among experiments are attributable to differences in food availability. Ref. 17 had the highest levels, this study was intermediate, and ref. 21 had the lowest levels.
† $P < 0.01$.
‡ $P < 0.05$.
§ $0.05 < P < 0.10$.
‖ Fish were only kept until they produced two litters of young in ref. 21.
Values for reproductive effort in ref. 21 represent a single estimate made at the end of the experiment; those for the other two studies represent the sum of four consecutive estimates. See ref. 17 for details on the latter analysis.

later visits to the field site, we found guppy populations to be large and widespread. Thus, subsequent genetic drift also seems unlikely.

Two years after the transfer, the phenotypes of females caught from the introduction site differed from those of females from the control site in offspring size and reproductive allotment (embryo weight/total body weight), suggesting a response to changed predation[13]. Six subsequent samples, collected between March 1981 and March 1988, revealed persistent differences between fish from these localities for many life-history variables (Fig. 1). Females from the introduction site produced larger offspring and initiated reproduction at a larger size than those from the control site. They also had smaller brood sizes and smaller reproductive allotments in the four dry-season samples (in (month/year) 3/81, 2/84, 4/87, 3/88), but not in the two wet-season samples (8/81, 6/83). We previously found, in a large-scale survey of many streams, that there was a similar trend towards lower reproductive allotment, lower fecundity and reduced differences between guppies from *Rivulus* and *Crenicichla* localities during the wet season[20]. Mature males from the introduction site were significantly larger than control site males prior to 1984, but smaller thereafter. With the exception of male size, the phenotypes of all variables over 11 years supports the predictions of life-history theory.

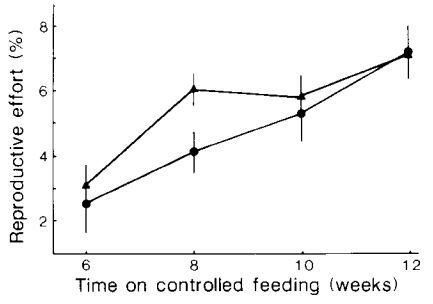

FIG. 2 Reproductive effort in the second generation, laboratory-reared fish at 6, 8, 10 and 12 weeks after the initiation of controlled food availability (following the methods of ref. 19). ▲, Downstream control; ●, Introduction site. Controlled feeding began when the fish were 25 days old. These time intervals correspond to routine changes in food availability. Earlier ages were not included because most females had not yet conceived their first litter. Later ages were not included because many females had already produced their third litter and were no longer in the experiment.

To determine whether these differences were heritable, we collected guppies from both localities in March 1987, then reared their descendants through two generations in a common environment, using the methods of our previous studies[17-19,21]. Rearing fish through two generations in a common environment eliminates environmental influences on the life history; remaining differences are assumed to have a genetic basis. Males and females from the introduction site mature at a significantly later age and larger size than those from the control site (Table 1); they also produce significantly fewer young in the first brood, but show no differences in the size of the second or third broods. This pattern of increased fecundity early in life corresponds to the results of laboratory selection experiments[10,25] and supports the hypothesis that early-life performance has a stronger influence on fitness than later-life performance[24-26].

Guppies from the introduction site produce larger offspring in all three broods, although the difference is only significant for the second brood. They have lower reproductive effort at 6, 8 and 10 weeks after the initiation of controlled feeding (Fig. 2), although the difference was only significant at 8 weeks. The differences in reproductive effort early in life are attributable to the delayed age of first parturition and smaller initial brood sizes in the introduction site guppies. All of these patterns match those observed among fish from two *Crenicichla* and two *Rivulus* sites considered in our earlier study[17] (Table 1). It is therefore clear that the introduction site guppies have evolved genetically new life-history parameters that correspond to the usual differences between these two types of localities.

The genetic differences in life histories reported here are far greater than those observed previously in a similar study[21] (Table 1); although both studies revealed equally strong differences in the life history phenotypes of wild-caught fish. The different results may reflect differences in the duration of the experiments (4 versus 11 years), and may illustrate the time required for a response to selection, arguing strongly for long-term field experiments. As these experiments are duplicates, the general similarity in their results, plus their convergence upon natural populations under the same predation regime, suggest that these are specific responses to natural selection, rather than the results of drift or founder effects.

These results demonstrate significant evolutionary changes in the life histories of introduction-site guppies, which are consistent with theoretical predictions. They provide direct experimental field evidence of the importance of predation in moulding life history evolution in guppies, though of course other factors may be important[21]. More generally, these results provide evidence that mean differences in age-specific survival will mould the evolution of life-history patterns. The widespread evidence for size-specific predation in other species[27-30] suggests that this could be a common factor in life-history evolution. □

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Formation of target-specific neuronal projections in organotypic slice cultures from rat visual cortex

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A CHARACTERISTIC feature of the mammalian cortex is that projection neurons located in distinct cortical layers send their axons to different targets. In visual cortex, cells in layers 2 and 3 project to other cortical areas, whereas cells in layers 5 and 6 project to subcortical targets such as the lateral geniculate nucleus[1,2]. The proper development of these projections is crucial for correct functioning of the visual system. Here we show that specific connections are established in an organotypic culture system in which rat visual cortex slices are co-cultured with another slice of the visual cortex or with a thalamic slice. The laminar origin and cellular morphology *in vitro* of cortical projections to other cortical regions or to subcortical targets are remarkably similar to those seen *in vivo*. In addition, axons of projecting cells are not restricted to particular pathways, but appear instead to grow directly towards their appropriate target. These observations raise the possibility that chemotropic attraction from the target areas may play an important part in the development of the cortical projection pattern.

Slices of rat visual cortex were co-cultured either with a slice from the lateral thalamus or another slice of visual cortex using a roller-culture technique[3]. We used animals from postnatal day 0 up to postnatal day 2, an age at which the axons of cortical projection neurons in rat have not yet reached their targets[4-6]. The slices were placed side by side less than 1 mm apart on glass coverslips and embedded in a plasma clot (Fig. 1*a*). As reported previously[7], cultures maintained in this manner flatten to one to three cell layers within about 10 days *in vitro*, contain all major cortical cell types and survive for several weeks. In the experiments described here, co-cultures were examined after 8-14 days *in vitro*.

As we were interested in the laminar origin of cortical projection neurons *in vitro*, we studied the layering of the cultured slices in detail. In the rat, the deepest cortical layer (sublayer 6B) forms a band of tightly packed cells lying immediately above the white matter[8]. This cell band was clearly visible in all Nissl-stained slice cultures and formed a sharp border with the white-matter zone (Fig. 1*c*). Usually, the remaining cortical layers could also be identified in cultures maintained for 1-2 weeks and were comparable to those in the normal visual cortex from animals of that age (Fig. 1*b*). The cortical layers of young animals, however, are less distinct than in the adult, and in slice cultures it was sometimes difficult to determine the exact boundaries of every layer. Therefore we used an additional approach to demonstrate the layering in our culture system, taking advantage of the fact that the cortical layers are born in an inside-first outside-last sequence[9,10]. Cells destined for different layers were labelled on their birth by injecting timed pregnant rats with 5-bromodeoxyuridine (BrdU; see Fig. 2 methods). We then prepared slice cultures from 0-2-day-old animals and compared the laminar distribution of labelled cells after 1-3 weeks *in vitro* with the location of labelled cells in littermates of the corresponding age. *In situ*, most cells generated on embryonic day 16 (E16) are located in the deep cortical layers 5 and 6, but cells generated on E18 are concentrated in the upper layers, 2 and 3 (ref. 11; Fig. 2*a* and *c*). In slice cultures, BrdU-labelled cells also formed a distinct band that was only slightly more scattered than *in vivo*. The laminar pattern was very much like the situation *in vivo* at the corresponding age (Fig. 1*d* and Fig. 2). Thus cortical cells in slice cultures are located at their appropriate laminar position.

The origin and cellular morphology of cortical neurons projecting to thalamic slices *in vitro* were determined by placing a small crystal of the lipophilic fluorescent dye DiI in the thalamic explant (see Fig. 3 methods). A micrograph and camera lucida drawing of these cells in a cortical culture are shown in Figs 3 and 4*a* respectively. All but one of the labelled cells were located in the deep cortical layers, precisely where cells projecting to the thalamus are found *in vivo*. In these experiments the thalamus was placed immediately adjacent to the white-matter side of the cortical slice. It is possible that only cells in the deep layers of the cortex, that is, those cells closest to the thalamus, are able to project to this target. To rule out this explanation we placed the thalamus next to the pial surface of the cortical culture, far away from the white matter. Again, axonal innervation of the thalamic explant originated from cells in the deep cortical layers. Cells in the superfical layers, although closer in distance to the thalamic explant, did not innervate this target (Fig. 4*b*). The axons of the deep cortical cells grew directly towards the thalamic explant. In many instances, axon collaterals separate from the main axon at right angles and run horizontally for

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