

Rapid evolution of a life history trait

(natural selection/copepod/diapause/fish predation)

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ABSTRACT The copepod *Diaptomus sanguineus* begins diapause in permanent ponds in late March as an adaptation to avoid summer fish predation. During a study of copepod populations in two Rhode Island ponds, a severe drought dried one pond killing all fish. The second (control) pond did not dry, and no fish were killed. Before the drought copepods in the two ponds entered diapause on nearly the same date. After the drought, the timing of diapause shifted to later in the year in the pond that had lost its fish, while no shift occurred in the control pond. The direction of this shift in the onset of diapause is that expected had the copepods been released from natural selection for early spring diapause imposed by summer fish predation.

There are surprisingly few examples of adaptive evolution by natural selection in wild populations. Indeed, the best documented examples of selection in the field are of population responses to human disturbance (1–3). Natural disturbances or catastrophes that might lead to evolutionary change are difficult or impossible to predict and so the locations of appropriate experimental and control areas cannot be anticipated nor can studies of initial conditions be planned.

Here we report observations involving two populations of the planktonic freshwater copepod *Diaptomus sanguineus* that were fortuitously under investigation when a natural disaster removed an important agent of selection from one population while leaving it unaltered in the other. The result was a rapid directional shift in the life history of the affected population while the control population remained unchanged.

STUDY SITES AND METHODS

Diaptomus sanguineus makes two kinds of eggs: (i) subitaneous eggs that hatch rapidly and (ii) diapausing eggs that rest in the sediments for an extended period before hatching (4, 5). Egg clutches are of one or the other type. Mixed clutches are never produced (5). The timing of the seasonal shift between egg types has been followed since 1979 for *D. sanguineus* populations inhabiting two small Rhode Island ponds. Bullhead Pond (2.3 ha, surface area; 4 m, maximum depth) and Little Bullhead Pond (0.1 ha; 2.4 m) lie in separate drainages within 200 m of each other on private land. In 1979 egg type was determined as described (4, 5) on groups of 20 ovigerous females collected biweekly. In subsequent years determinations were made on groups of 24 ovigerous females collected weekly, except in 1980 when no determinations were made.

To ascertain the extent to which photoperiod controls *D. sanguineus* reproductive phenology, we collected first stage nauplii (newly hatched) in the field and reared them to sexual maturity (about 30 days) in the laboratory in four controlled environment chambers at $9 \pm 1^\circ\text{C}$. Photoperiods were chosen

(Table 1) to bracket the day length at the time of year that most copepods begin producing diapausing eggs.

RESULTS AND DISCUSSION

In Bullhead Pond there is a sharp switch from subitaneous eggs to diapausing eggs at the end of March (Fig. 1A). The repetition of this phenomenon in all 5 yr of study suggests that it is controlled proximately by some highly repeatable environmental cue such as day length (6). Laboratory experiments described below support this interpretation.

Hairston and Munns (4) present evidence that the timing of this switch is an adaptation to avoid seasonally intense predation by sunfish. Earlier work (7) showed that as water temperatures increased in the spring, planktivory by fish also increased, resulting in selective mortality of the more visible female copepods, especially those carrying eggs. While subitaneous eggs cannot survive passage through the digestive system of a fish, diapausing eggs can be collected from fish feces and hatched (5). In Bullhead Pond, diapausing eggs develop and hatch in November and December after the fish have become quiescent. The copepods are active during winter and spring, but in summer are only present as diapausing eggs in the pond sediments. Using the timing of the spring sex ratio increase as a measure of the initiation of fish predation, Hairston and Munns (4) showed that the springtime switch to diapause in Bullhead Pond occurred on almost exactly the dates expected if fish predation were assumed to be a seasonal catastrophe that only diapausing eggs could survive.

A severe drought in 1980 caused the water level of Little Bullhead Pond to drop from 2.4 m to 0.2 m, and in January 1981 it froze completely, killing all 13,900 resident sunfish (7). After fluctuating several times in 1981 between 0.4 m and total dryness, the pond refilled in January 1982. During the drought, the depth of neighboring Bullhead Pond dropped from 4.0 m to 1.5 m, but no fish mortality was observed.

These occurrences at Bullhead and Little Bullhead Ponds represent a natural fish-removal experiment that released the copepods in Little Bullhead Pond from an important source of natural selection. Without predators, copepods switching to diapause later in the season should have been favored, because individuals whose eggs hatch immediately produce more total descendants in a year than their diapausing counterparts (4).

In 1979, when fish were present, the switch to diapause occurred in Little Bullhead Pond at essentially the same time as in Bullhead Pond (Fig. 1B, Kolmogorov–Smirnov test, $P > 0.5$). Thus the initial conditions for diapause in the two ponds were similar. In November 1982, *D. sanguineus* hatched from diapausing eggs in Little Bullhead Pond and developed according to the pattern typical of Bullhead Pond copepods. Yet when these copepods matured in 1983, they did not switch from making subitaneous eggs to diapausing eggs until mid-April (Fig. 1B), nearly a month later than the switch time observed for copepods in Bullhead Pond that same year (Fig. 1A, the difference is significant by

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Table 1. Fraction of female *D. sanguineus* from Bullhead Pond making diapausing eggs when reared in the laboratory at four photoperiods

Photoperiod, hr:min (light/dark)	Fraction diapausing	Sample size of females
9:55/14:05	0.15	72
11:40/12:20	0.62	131
13:05/10:55	0.86	98
14:45/ 9:15	0.96	48

The photoperiod at the mean date of the switch to diapause in the field (24 March) is 12:15/11:45 (sunrise to sunset).

Kolmogorov-Smirnov test, $P < 0.001$). This striking shift in the timing of diapause in just two generations is in the direction expected of an adaptive evolutionary response to fish removal.

The year after the drought, planktonic predatory midge larvae, *Chaoborus americanus*, appeared in small numbers in Little Bullhead Pond. These large and relatively visible insects are excluded from lakes containing planktivorous fish (8), and apparently immigrated (the adults fly) to the pond after the fish-kill [*C. americanus* was abundant in several nearby fishless temporary ponds (6, 9)]. In subsequent years they reached progressively greater abundances, so that in 1984 they were present at the extraordinary density of one larva per liter. The planktonic midges are, themselves, voracious predators, and in 1984 they completely consumed the developing cohort of *D. sanguineus*. Thus the natural experiment ended naturally in 1984. The *Chaoborus* could not have caused the change in diapause timing, however, because predation from them was most intense in winter when they were present as fourth instars and, if anything,

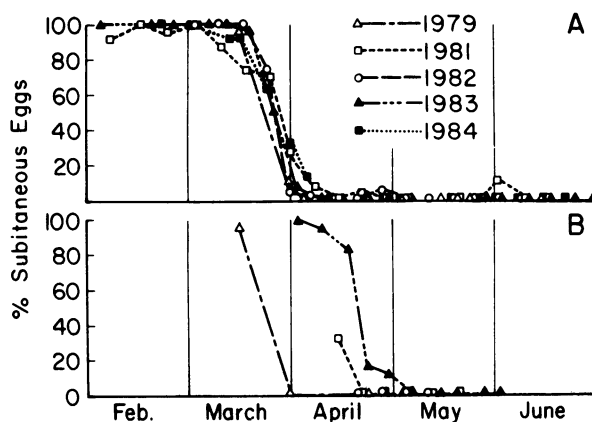


FIG. 1. Percentage of ovigerous female *D. sanguineus* carrying immediately hatching (subitaneous) eggs, as distinct from diapausing eggs, in two ponds in several years. (A) Bullhead Pond population where fish remained present throughout the period of the study. (B) Little Bullhead Pond population where fish were present in 1979 and absent in 1981, 1982, and 1983. No data were collected in 1980. Drought years were 1981 and 1982. Diapausing eggs routinely hatch in Bullhead Pond in November and December and mature in January or February. This was also the case in Little Bullhead Pond in 1978 and 1979, but in 1981 diapausing eggs did not hatch until March when the ice melted, and in 1982 they did not hatch until February after the pond refilled at the end of the drought. In these years, the *D. sanguineus* did not mature until mid-April, well past the time that the copepods in Bullhead Pond (and Little Bullhead Pond, 1979) had switched from subitaneous to diapausing egg production. As a result, nearly all copepods collected from Little Bullhead Pond in 1981 and 1982 made diapausing eggs, presumably because they began reproducing only after day length exceeded the critical photoperiod cuing the late-March switch in egg type. In these years, with fish absent, any female making subitaneous eggs first and switching to diapause later in the season would have gained a large reproductive advantage.

would have selected for substantially earlier rather than later diapause.

These observations have the shortcomings typical of natural experiments: imperfect control, a question as to exactly what experiment nature has carried out, and a lack of replication. Although these can be important, they are significantly ameliorated in the present case by a feature frequently missing from other natural experiments: the initial conditions are known. The basic patterns of diapause and predation-induced mortality were the same in both ponds before the drought (7), even though the control pond is larger and deeper than Little Bullhead Pond. During the period of freezing and drying, the copepods survived in the sediments as diapausing eggs. Since *D. sanguineus* is also a resident of temporary ponds in Rhode Island (5, 6, 9), these are not novel stresses for the species. In addition, the chemical condition of the pond appears to have been little altered by the drought. For example, it is unlikely that there was any accumulation of salts as water level dropped, because the pond sits on a gravel moraine and fluctuates in depth with the water table. In fact, it is difficult to imagine a more ideal fish-removal method than the one that occurred naturally.

How did the response occur? Three possibilities exist. First, the copepods may have somehow sensed the absence of fish and adjusted their reproduction accordingly. This is not an unreasonable suggestion since a variety of zooplankton species have been shown to respond morphologically to the presence of chemical compounds exuded by their predators (10–12). However, Hairston and Olds (5) found in a reciprocal transfer experiment of *D. sanguineus* between Bullhead Pond and a fishless temporary pond, that regardless of pond type, females switched between subitaneous and diapausing eggs as if they were in their home pond. In contrast, individual female copepods reared in the laboratory repeatedly responded to changes in the photoperiod to which they were exposed by altering the type of eggs they produced (N.G.H., Jr., and E. J. Olds, unpublished personal observation). Thus, although the copepods are capable of responding reproductively to one type of environmental change (day length), they apparently could not sense a change in pond type (and hence predator presence) and respond accordingly. A second possibility is that all of the Little Bullhead Pond copepods were exterminated in the drought and replaced after the pond refilled by migrants from another population with a different diapause phenotype. This suggestion can be rejected because after the drought, immature *D. sanguineus* were reestablished in the plankton by the hatching of diapausing eggs. The first generation after the drought reached 1.6×10^6 individuals. Dispersal among ponds is at best infrequent (13, 14) and could not produce the numbers seen here. There are no streams flowing into or out of Little Bullhead Pond by which transport might have occurred. The only other reasonable possibility is that the timing of diapause shifted in the population as a result of natural selection.

How plausible is the natural selection explanation? One approach is to treat the data as a selection experiment on a polygenic trait [there exists considerable evidence for polygenic inheritance of diapause characteristics in insects (15)]. The fraction of the copepods switching egg types between successive sampling dates can be plotted as a frequency distribution of the phenotypic expression of diapause timing (Fig. 2). The number of days by which the mean switching date moved from 1979 to 1983 can be considered the response R to natural selection for delayed diapause (16), where $R = h^2S$, and h^2 is the heritability of diapause timing; S is the selection differential, the difference between mean switching dates of the entire starting population and those individuals contributing to future generations.

Do reasonable combinations of h^2 and S exist for the response observed? Sampling at Little Bullhead Pond was in

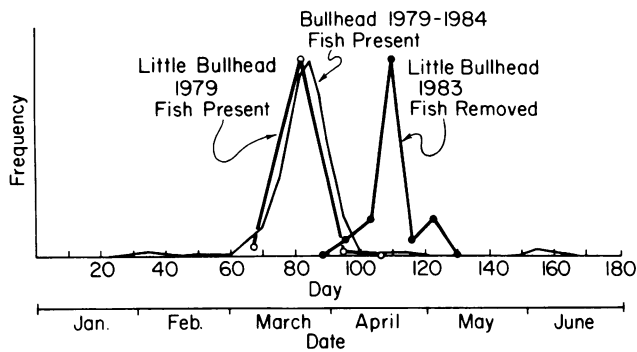


FIG. 2. Frequency distributions showing the fractions of *D. sanguineus* populations switching from making immediately hatching (subitaneous) eggs to diapausing eggs before (1979) and after (1983) fish removal in Little Bullhead Pond. Also shown is a composite distribution for Bullhead Pond constructed by summing frequency distributions from 5 yr (1979, 1981–1984) of data shown in Fig. 1A. The fraction switching between two consecutive sampling dates is plotted at the intermediate date. The scale for the abscissa is given as both Julian days and calendar dates.

fact too infrequent in 1979 to establish the distribution of times of copepod diapause for the low frequency phenotypes that would be important here. However, it seems reasonable to use the 5 yr of data from Bullhead Pond as a control population to characterize what the Little Bullhead Pond population was like before its fish were killed. These data produce a distribution with a mean on day 84 (24 March) and two extended tails that indicate extensive character variation upon which selection might act (Fig. 2). Taking these data as the distribution of diapause timing with fish present and Little Bullhead Pond 1983 as the response, the mean switching date moved from day 84 in the control to day 110 (20 April 1983) after fish removal, or by $R = 26$ days. The response took place over 2 yr, and two copepod generations, during which time selection for delayed diapause is assumed to have acted. Since response to selection should be linear with time (ref. 16, page 185), we need only explain $R/2$ or 13 days of response per generation. Heritability is not known in this instance, but ranges in theory between zero and one. Values for a broad variety of species and characters summarized by Falconer (ref. 16, page 150) range from 0.05 to 0.70. By assuming an intermediate value of 0.5, we obtain $S = 26$ days or a mean switching date for the selected individuals of $84 + 26 =$ day 110 (20 April). Since this date falls within the distribution of the control population, natural selection may have produced the result observed. Heritabilities as low as 0.33 allow for reasonable S values (≤ 39 days) that include a substantial proportion ($\geq 5\%$) of the initial population size. This last figure, incidentally, does not imply that copepod population size must have been reduced by a factor of 20 during the period of selection (1981–1983). *D. sanguineus* has two generations per year, one in midwinter and one in the spring. Selection for diapause only occurs in the spring generation, and any resulting reductions in density due to genetic load could readily be made up by reproduction in the winter generation. In winter each female produces up to five clutches, each containing 40–60 eggs.

It is a bit difficult to know what h^2 value should be viewed as plausible here, because literature estimates of heritability frequently have extremely large associated standard errors (ref. 16, with coefficients of variation reaching 150–300%, e.g., refs. 17–19). However, there is at least evidence of a strong genetic component to the diapause timing trait in *D. sanguineus*. Copepods reared from hatching to adulthood

under identical constant environments showed remarkable interindividual variability in the type of eggs produced (Table 1). This result suggests that the variation seen in the timing of the switch to diapause in the field (Fig. 2) is to a significant extent the product of innate variation between copepods in their photoperiodic responses.

The assumptions contained in the calculations above are restrictive (16, 20), and there is disagreement about the robustness of the method when these restrictions are violated. Since we consider here only whether natural selection can have produced the phenotypic change observed, it may be sufficient simply to note that the mean timing of the switch to diapause after fish removal lies within the distribution of phenotypes present in the control population.

Character response to strong directional selection is undoubtedly an uncommon occurrence in natural populations, yet it is under this condition that adaptive evolution is most easily observed. There are few reports of directional phenotypic changes in a species following an extreme environmental deviation (21). In the present case, not only were an environmental change and a population response recorded, but the study includes a control population that did not change. The resulting data document what appears to be a rare example of adaptation by natural selection acting on a wild population.

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