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Population differences in the timing of diapause: adaptation in a spatially heterogeneous environment

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Summary. Populations of the planktonic copepod, *Diaptomus sanguineus*, live in permanent and temporary freshwater ponds in Rhode Island. All ponds in which they occur become uninhabitable at some time during the year, but the nature and timing of the harsh period varies both spatially and temporally. Females produce discrete clutches either of subitaneous eggs which hatch immediately or of diapausing eggs which hatch the following season. The two egg types show distinct chorion morphologies under transmission electron microscopy. In permanent ponds the copepods start making diapausing eggs in March, one month before rising water temperatures induce planktivorous sunfish to become active. In temporary ponds diapausing eggs are produced, in a complex pattern from May to July, before the water disappears in late summer or early fall.

We investigated the spatial scale at which *D. sanguineus* is adapted to this complex environment. In a reciprocal transfer experiment between temporary and permanent bodies of water, female copepods placed in new ponds made subitaneous and diapausing eggs in the same sequence as control females retained in their home ponds. The copepod populations enter diapause at times appropriate for the local habitat conditions they experience, but inappropriate for other, nearby ponds. Transplanted females were unable to sense a change in pond type or to adjust egg production accordingly. We conclude that *D. sanguineus* populations are adapted to the specific conditions of isolated ponds rather than to a broader geographical region containing several pond types.

Introduction

Wholly aquatic organisms inhabiting lakes and ponds experience a particularly interesting regime of natural selection. Depending on the extent of habitat differences and on the rates of transport of individuals between bodies of water, adaptation may occur narrowly to the specific conditions of isolated localities, or more broadly to the average conditions prevailing over some geographic region. Levins (1968) developed this idea in his discussion of environmental "grain." He was principally concerned with whether an individual encounters a single (coarse-grained) environment, or several types of (fine-grained) environments during its life time, but his approach is also useful in general considerations of the spatial scale at which adaptation occurs. The important effects of migration and dispersal on adaptation have recently been further elucidated for populations living in environments with multiple niches (e.g. Gillespie and Langley 1976; Gillespie 1977) and along clines (e.g. Endler 1977).

One of the adaptations that Levins (1968) discussed in some detail was diapause. When the environment becomes uninhabitable at certain times of year, organisms with no means of active dispersal may enter a dormant state resistant to the harsh conditions. He pointed out that the timing of the switch to diapause must depend on generation time, which ultimately affects whether an individual encounters both the harsh and the favorable periods, or is only alive long enough to experience one. Thus environmental grain may take on either spatial or temporal dimensions; or even both, as when differences exist in the timing of the seasonal catastrophe from one locality to another. It is in this last most challenging environment that the freshwater calanoid copepod, *Diaptomus sanguineus*, resides.

In southern Rhode Island the habitats of *D. sanguineus* include both temporary and permanent ponds. In temporary ponds, harsh conditions begin in late summer as the water disappears, whereas in permanent ponds spring-time increases in feeding activity by dense populations of sunfish make the limnetic zone uninhabitable (Hairston et al. 1983). *D. sanguineus* disappears from the water at the onset of the harsh conditions, and reappears in the winter when the temporary ponds refill and planktivory in the permanent ponds declines. We have established, in the four ponds we have studied, that the copepods avoid uninhabitable periods by producing diapausing eggs that rest on the bottom until conditions ameliorate (Hairston and Munns 1984, and this report). Diapause begins in the permanent ponds abruptly at the end of March, whereas in the temporary ponds it begins variably between late May and early July.

The likelihood of transfer of individuals between ponds on the feet, fur or feathers (Thienemann 1950; Löffler 1964) or in the stomachs (Mellors 1975) of vertebrates is possible, but probably a rare event. The distances between the four ponds range from 100 m to 27 km. Yet, we have been unable to discern any differences in external morphology (body size or sexual characters) between the four populations that would indicate reproductive isolation. What then is the basis for the differences in the timing of diapause between the four populations? Three possibilities exist. (i) Diapause in each case might be adapted to a specific, local

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selective pressure, (ii) the copepods might in some way sense the type of pond they are in and adjust diapause accordingly, or (iii) the copepods might follow some general, all-purpose diapause strategy that works reasonably well everywhere, but not perfectly anywhere.

Here, we describe the nature of diapause in *D. sanguineus*, and report the results of a reciprocal transfer experiment in which adults were placed in small enclosures and exchanged between a temporary and a permanent pond. Females were monitored to establish the time at which they switched from making immediately hatching (subitaneous) eggs to making diapausing eggs. The experiment permits us to ask if diapause is a local, coarse-grained adaptation or a regional, fine-grained adaptation.

Methods

Study organism and study sites

Diaptomus sanguineus is a small (metasome length ca. 1.0 mm) calanoid copepod broadly distributed across the northern United States and Canada, extending south to Virginia and down the Mississippi Valley to the Gulf of Mexico (Wilson and Yeatman 1957). The females carry their eggs in a sac attached to the genital segment. Subitaneous eggs are those that are carried until they hatch (about 4 days at 15° C), whereas diapausing eggs are carried for two or three days and then dropped to the bottom of the pond where they rest until hatching the following year. Females produce sequentially up to four clutches, but apparently cannot store sperm since insemination by a male is required between clutches (this study, Watras 1980). Clutches of mixed egg types are never produced. The eggs hatch as nauplii and pass through six naupliar instars and five copepodid instars before becoming mature in the sixth copepodid stage.

Collections and experiments were undertaken at two ponds in Rhode Island. Bullhead Pond is a spring-fed, permanent body of water without inflow or outflow streams. It is located 3 km west of Perryville, South Kingstown, R.I., has a surface area of 2.3 ha and a maximum depth of 3 m. It contains about 30,000 sunfish (*Lepomis marcochirus*, *L. gibbosus*, and *L. auritus*), or about one fish for every two cubic meters of water (Hairston et al. 1983). Pond A is temporary and therefore has no fish. It receives runoff in the spring from an ephemeral stream, but during the rest of the year has no overland input. It is located at the W. Alton Jones Campus of the University of Rhode Island, West Greenwich, R.I., has a surface area of 0.25 ha and a maximum depth of 2 m when full. The two ponds are 27 km distant from each other.

Zooplankton collection and analysis

Duplicate zooplankton samples have been collected weekly at both ponds since 1978. When the ponds were ice-free, we made routine collections using a Clarke-Bumpus sampler fitted with a 75 μm-mesh net. From December to February in most winters, Bullhead Pond was covered with ice, making sampling difficult. In the winter of 1980–1981 zooplankton samples were collected through a hole in the ice using a hand pump and a 75 μm-mesh net. Estimates of copepod abundance using the latter technique are probably unreliable because they are highly susceptible to patchy distribution within the pond, however they do serve to show when different copepod instars were present. In Clarke-Bumpus hauls, copepods were collected throughtout the entire pond integrating any fine scale differences in distribution. Plankton were preserved in 10% Formalin, returned to the laboratory and counted in a Bogorov tray at 40X.

Diapausing and subitaneous eggs of *D. sanguineus* are difficult to distinguish under light microscopy. This is true for other diaptomid copepods as well (Brewer 1964; Cooley 1971; Watras 1980), and our method for determining egg type was the same as that used by previous investigators. Females carrying egg sacs were isolated in small vessels, and egg development was followed for at least two weeks. Eggs that had not hatched at the end of this period were designated diapausing eggs. To check the reliability of this method, we observed for six months, 100 egg sacs that had not hatched. They were kept in the dark at 4° C. None hatched during this period, but 95% were induced to hatch by exposure to low dissolved oxygen (accomplished by bubbling nitrogen gas in the water). Thus, we determined that these were diapausing and not dead eggs.

Transmission electron microscopy

Egg types could also be distinguished by electron microscopy. Egg sacs designated subitaneous or diapausing by the technique just described were separated from live females and fixed over night in 2.5% gluteraldehyde in 0.05 M PO_4 buffer, pH 8.0. After embedding in 1% agar, the sacs were washed in buffer and post-fixed for one hour in 1% OsO_4 in pH 6.8 PO_4 buffer. They were then dehydrated in acetone, and embedded in Spurr's low viscosity medium in aluminum weighing dishes. Thin sections were mounted on 100-mesh Formvar coated grids, stained with uranyl acetate and lead citrate and examined with a Hitachi HS-9 transmission electron microscope at 75 kV.

Reciprocal transfer experiment

At each pond on each sampling date in 1982, 48 live female *D. sanguineus* carrying eggs were placed singly in 15 ml wells of four 12-well plastic tissue culture plates, filled with 75 μm-mesh filtered pond water. The wells on each plate were then covered with a single piece of 75 μm-mesh polyester netting, held down by the plate's plastic lid, in which holes had been cut to correspond with the positions of the wells. The entire plate with lid, netting, water and copepods, was held tightly closed with four rubber bands. Each plate therefore had an array of 12 females carrying eggs, each in a convenient volume of water with a fine mesh window for exchange with the pond environment. Copepods were placed in the plates in the field, and immediately two of the plates were resuspended in the pond from which they were taken. The other two plates were transported in an ice chest at pond temperature to the second pond and suspended in the water there.

For suspension in the ponds, the plates were secured in an aluminum rack made to hold 20 plates flat, without stacking. Racks were hung at mid-depth in the ponds from anchored styrofoam floats, so that the plates faced sideways, facilitating water exchange through the netting. The float acted somewhat like a sail gently moving the racks when the wind blew. An experiment using crystal-violet stain showed that 25% of the water in each well exchanged with the pond every hour, or put another way, only 37%

of the water originally in a well was left after four hours. Thus, communication between the pond and the wells was fairly rapid.

Each plate was checked under a stereomicroscope twice per week. Females whose eggs had hatched or that had dropped diapausing eggs were moved to a new well and paired with a male from the female's "home" pond. This was continued until the female died. In this way, we were able to establish the sequence of egg types produced by individual females through time, both in their home ponds and in the ponds to which they were transferred. By the end of the experiment, we knew the reproductive sequences of 1,778 female copepods from either Pond A or Bullhead Pond.

Results and discussion

Population dynamics

The production and hatching of diapausing eggs and their impact on the dynamics of the *D. sanguineus* populations may be seen in cohort analyses from the two ponds. At Bullhead Pond in 1980 when samples were taken through the ice, diapausing eggs hatched and appeared in the water column as first stage nauplii in late autumn (Fig. 1). The cohort matured in February and March and the females made eggs which were at first exclusively subitaneous. These eggs gave rise to a second generation that matured in April and May. In late March, the entire population switched from producing subitaneous eggs to producing diapausing eggs. This occurred at the end of the first generation, and just as individuals from the second generation began to reach the adult stage. The switch was not the result of first generation females making subitaneous eggs and second generation females making diapausing eggs because individual females followed during this period made subitaneous eggs early in life and diapausing eggs later (see below). Diapausing eggs appear in Fig. 1 as a distinct group which did not give rise immediately to a third generation. Rather, they disappeared from the plankton and rested on the bottom until the next autumn.

We followed the onset of diapause in Bullhead Pond in four years, and in each case females started making diapausing eggs in late March (Hairston and Munns 1984). Sunfish began to have a measurable impact on copepod mortality in each of 5 years in late April or early May (Hairston et al. 1983). By competing model populations in a computer simulation, Hairston and Munns (1984) found that copepods diapausing 1.2 to 1.4 generations before the average date of increased fish feeding would have a competitive advantage over those diapausing either earlier or later in time. In Bullhead Pond, the late March diapause of *D. sanguineus* falls during the expected period, 1.3 generations before the mean date of the beginning of increased fish activity.

In 1982, the *D. sanguineus* in Pond A went through three complete generations. The pond filled with melt water in January and diapausing eggs hatched soon after. The first generation was not detected (Fig. 2) until it had reached the early copepodid stages due to the difficulties of sampling through thin ice. It matured in March and April and made principally subitaneous eggs. The first individuals of the second generation reached adulthood in May at a time when the population was switching to making diapausing eggs. Then, as the second generation adults began to decline in abundance, the population switched back to producing subitaneous eggs. These gave rise to a third generation which made only diapausing eggs when it matured in July and August. Pond A was completely dry by mid-September.

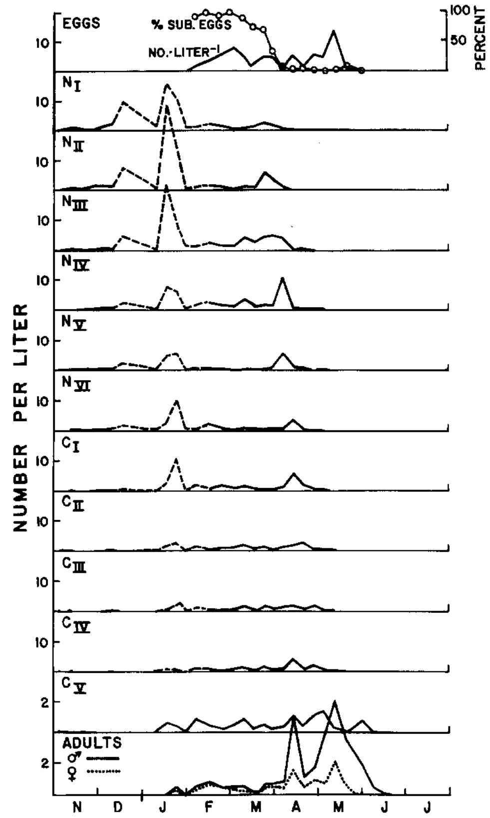

Fig. 1. Cohort analysis of *Diaptomus sanguineus* in permanent Bullhead Pond, 1980–1981. The abundance of each life history stage is plotted against date showing two discrete generations. Naupliar stages designated *NI* through *NVI* and copepodid stages *CI* through *CV*. Note the scale change for fifth stage copepodids and adults. The dashed lines denote samples taken during the winter through the ice. Also shown is the percentage of clutches that were subitaneous (immediately hatching) eggs

There are two possible explanations for the switch back to subitaneous eggs in June. The computer model of diapause suggests that when the time of the onset of uninhabitable conditions in Pond A is highly variable from year to year, the competitively superior strategy is for each female to make a mixture of subitaneous and diapausing clutches regardless of her proximity to the end of the season. With this "bet-hedging" strategy a female would have some offspring growing up to reproduce in the event that conditions remain favorable and others diapausing in case conditions deteriorate. The date of drying of Pond A varies broadly between early August and early October (from 13 years of data collected by C.R. Shoop, pers. com.), and in 1979 it retained water year-round. Were *D. sanguineus* in Pond A pursuing the bet-hedging strategy, the population would switch back and forth between the production of subitaneous and diapausing eggs as each generation matured, as seen in Fig. 2. The second explanation is that in mid-June 1982, Rhode Island received an unusual amount of rain which refilled Pond A and hence delayed its drying by about a month. Were *D. sanguineus* somehow able to sense this and adjust its reproduction accordingly, the return to subitaneous eggs might represent a close tracking of the pond environment. We cannot assess the relative merits of these two very different explanations with the data available.

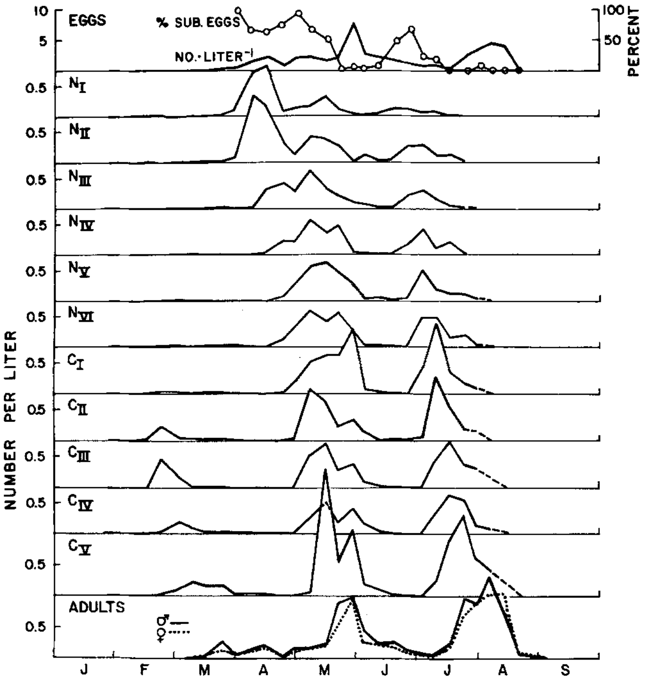

Fig. 2. Cohort analysis of *Diaptomus sanguineus* in temporary Pond A, 1982. The abundance of each life history stage is plotted against date showing three discrete generations. Naupliar stages designated *NI* through *NVI* and copepodid stages *CI* through *CV*. Because a second species of copepod appeared in August with immature stages indistinguishable from *D. sanguineus*, dashed lines at the end of the third generation are used to represent reasonable extrapolations of instar abundances. Also shown is the percentage of clutches that were subitaneous (immediately hatching) eggs

Transmission electron microscopy

Subitaneous eggs of *D. sanguineus* are covered with a shell of homogeneous material 0.3 μm to 0.6 μm thick (Fig. 3A). In contrast, diapausing eggs have a much thicker shell, 1.7 μm to 3.9 μm thick, consisting of an outer electron dense cortex and a distinct, less dense inner layer (Fig. 3B). Both layers are characterized by a series of fine lamellae about 0.1 μm thick. Champeau (1970) described a nearly identical structure for diapausing eggs of *Hemidiaptomus ingens provinciae*. An apparently analogous structure (Mazur pers. com.) has been described in detail for silk moth chorion (Mazur et al. 1982). The lamellar arrangement is produced by a helicoidal array of fibers which, due to their regularly changing orientation in the plane of sectioning, give the appearance of banding (Bouligand 1972). The structure is typical of the integument of many arthropods including adult harpaticoid copepods (Gharagozlou-Van Ginneken and Bouligand 1973).

Egg types distinguished by the hatching procedure also show striking differences in egg shell structure by electron microscopy. The thicker shell of diapausing eggs not only protects them from desication in temporary ponds but also from digestion by fish in permanent ponds. Diapausing eggs survive gut passage whereas subitaneous eggs do not (Hairston and Munns 1984). Further, the structural differences provide a reasonable hypothesis to explain our observation that female *D. sanguineus* do not make clutches of mixed egg types. The separate physiological processes involved in laying down such distinct shells presumably constrain an individual to make only one type at a time, and impose an interesting limit on the variety of diapause strategies available to the copepods.

Reciprocal transfer experiment

Figures 1 and 2 show that while both *D. sanguineus* populations go through a series of generations beginning and ending with diapausing eggs, the time of year that the populations are present and the pattern of the switch to diapause are strikingly different. These differences afford us the op-

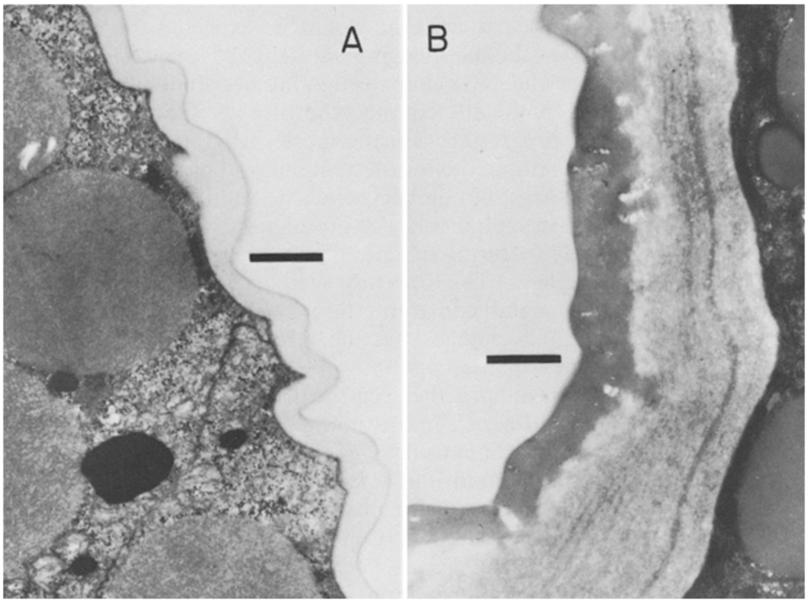

Fig. 3A. Transmission electron micrographs of sections through **A** subitaneous (× 11,875) and **B** diapausing (× 11,900) eggs of *Diaptomus sanguineus*. Scale markers = 1 μm

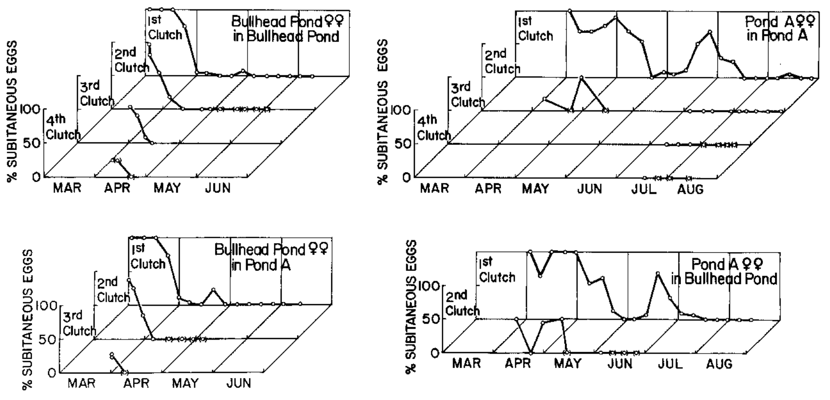

Fig. 4. Percentage of clutches of subitaneous (immediately hatching) eggs of *Diaptomus sanguineus* as a function of data for females from permanent Bullhead Pond and temporary Pond A. Copepods were either retained in the pond of origin or transferred as indicated in the four panels

portunity to investigate the spatial scale at which the adaptation for diapause is selected in *D. sanguineus*. Does it possess adaptations appropriate for the pond in which we collected it but inappropriate for other, nearby ponds? Can the copepods adjust their reproductive physiology in response to changes in pond type? Or do they follow the same sequence of diapause in all ponds, perhaps tied to copepod age rather to time in the season, representing a compromise adaptation to a patchy environment?

The results of the reciprocal transfer experiment are illustrated in four graphs in Fig. 4. Each point represents, for the 24 gravid females collected on the date shown, the percentage of clutches that were subitaneous and hatched during the two-week period we observed them. As previously described, the rest of the clutches are taken to be diapausing eggs. The points along the top line in each graph give the composition of clutches carried by the females at the time that they were collected. Points on successively lower lines give the composition of second, third and fourth clutches produced by females after their capture. The points on these lower lines are plotted at the mean dates on which they were produced. Thus, in the graph at the upper left, 100% of the female *D. sanguineus* collected and resuspended in Bullhead Pond on 2 March, 1982 were carrying subitaneous eggs. Ninety five percent of their second clutches, that is their first produced in the enclosures, were subitaneous eggs and 5% were diapausing eggs. Their third and fourth clutches were 53% and 25% subitaneous, respectively. In the graph at the lower left, 100% of females collected in Bullhead Pond and resuspended in Pond A on 2 March, 1982 were carrying subitaneous eggs. Eighty eight percent of these females made subitaneous eggs for their second clutches, and 25% made subitaneous eggs for their third clutches.

During the course of the experiment, females kept in the tissue culture wells suffered mortality rates between 0.05 and 0.25 per day; about the same rates as those calculated for females living free in the ponds (Hairston et al. 1983). Thus estimates of successive clutch types produced by groups of females collected on any given date are based on successively fewer individuals. Data points in Fig. 4 enclosed in parentheses are estimates derived from fewer than five females, and tend to occur in later clutches as females grew old, or late in the season when the females were old at the time they were collected. For unknown reasons, females from Pond A experienced much greater mortality in both ponds than did females from Bullhead Pond. The exception to this was the survival of third generation females kept in Pond A (Fig. 4, upper right, July and August). The cause of mortality was not starvation since parallel experiments with females in wells enriched with *Scenedesmus* for food showed similarly high mortality, nor was oxygen depleted in the wells since measurements with a picoammeter gave values comparable to those in the ponds (7.5 to 8.0 ppm).

The dates that the two populations switched to making diapausing eggs are accurately represented in the top line of each graph, since these were the clutches carried by females at the time of collection. Bullhead Pond females retained in Bullhead Pond switched to making diapausing eggs at the same time or only slightly later than these "first" clutches, indicating that the cue for switching egg types was not strongly affected by experimental conditions. For Bullhead Pond females transferred to Pond A the interclutch interval was somewhat longer and fewer clutches were produced than for those retained in Bullhead Pond, probably because the water in Pond A was about 4° C colder than in Bullhead Pond.

Second, third and fourth clutches from Pond A females retained in tissue culture wells in either pond did not follow a pattern of diapause similar to that seen in the pond itself (Fig. 4, 1st clutch). Rather than showing the consistent switch from subitaneous to diapausing eggs seen in all clutches from Bullhead Pond females, later clutches from Pond A females were more frequently composed of diapausing eggs; a pattern that would be expected for copepods following the bet-hedging strategy of early subitaneous clutches and later diapausing clutches. Individual females in each pond altered the type of eggs they made as the season progressed. Furthermore, females switched only from making clutches of subitaneous eggs to making diapausing eggs. No female switched the other way. The June increase in subitaneous egg production in Pond A was made by newly maturing females that subsequently made all diapausing eggs. This one-way switch may represent another developmental constraint (along with that of single egg types in all clutches) on the array of diapause strategies available to *D. sanguineus*.

The results of the reciprocal transfer experiment illustrate the difference in the timing of the switch from subitaneous eggs to diapausing eggs made by female *D. sanguineus* in Bullhead Pond and Pond A, and show that copepods transferred to a new locality continued to make diapausing eggs as if they had not been manipulated. In March, when Pond A females had not yet matured, Bullhead Pond females started out making subitaneous eggs, but gradually switched to making diapausing eggs. Bullhead Pond females placed in Pond A followed the same switch to diapause as those kept in Bullhead Pond. In April and early May, while Pond A females were producing principally subitaneous eggs, Bullhead Pond females suspended in Pond A made diapausing eggs. At the same time in the reciprocal treatment, 50% of the second clutches made by Pond A females in Bullhead Pond were subitaneous eggs while Bullhead Pond females retained in Bullhead Pond made almost exclusively diapausing eggs.

The differences in diapause observed between populations of *D. sanguineus* may well have a genetic basis. Reports of latitudinal variation in the timing of diapause both between closely related species and within species of insects are not uncommon (e.g. Walker 1980; Dingle 1981; Showers 1981), and intrapopulation variation in diapause is the foundation for laboratory selection experiments altering its timing and intensity (e.g. Hoy 1978; Showers 1981). Similarly, latitudinal differences in the timing of the production of diapausing eggs have been noted in freshwater calanoid copepods, but manipulative experiments have not been carried out. Populations of *Diaptomus denticornis* in northern Europe make only diapausing eggs, whereas those from southern Europe make only subitaneous eggs (Hutchinson 1967). Latitudinal variation of life history characters other than diapause have been shown to have a genetic basis in the freshwater cyclopoid copepod *Mesocyclops edax* (Allan 1982; Wyngaard 1982).

We are only now investigating the mechanisms by which the switch to diapausing egg production is effected. The fact that the time of the switch is the same for females retained in their native ponds or transferred to foreign ponds suggests that any environmental cue involved must be consistent from habitat to habitat. One such mechanism, photoperiod, has been established as an important cue for diapause or dormancy in a great variety of organisms (e.g. Harper 1977; Dingle 1978; Beck 1980; Flint et al. 1981) including the marine calanoid copepod *Labidocera aestiva* (Marcus 1980, 1982). Circumstantial evidence for a population of *D. sanguineus* living in Little Bullhead Pond, R.I. points to the involvement of photoperiod, but laboratory experiments under controlled conditions will be required to test this hypothesis.

Diaptomus sanguineus lives in a spatially and temporally heterogeneous environment. Some of this environmental variability is predictable: all ponds become uninhabitable for *Diaptomus* at some time of year, fish in permanent ponds become active in the spring, and temporary ponds tend to dry up in mid- to late-summer. Other variabiltiy is not predictable: there is year to year variation in the length of winter and the timing of the increase of fish activity; there is greater variation in summer rainfall and the length of time that temporary ponds contain water; and copepods transported by vertebrates from one pond to another may experience radically and unpredictably different environments. Female copepods begin making diapausing eggs at different times of year in different ponds. In the introduction we proposed three alternative explanations for this observation, each related to the environmental grain or spatial scale at which the copepods might adapt to their environment. The possibility of course remains that the differences in diapause between the two populations do not represent adaptations of any kind, and are instead the product of genetic drift in isolated populations. However, diapause critically controls the number of offspring surviving to the next season, and therefore is particularly likely to be subject to natural selection. In addition, the fact that Bullhead Pond copepods switch to diapause within a very few days of the date predicted to be the evolutionarily stable strategy (Hairston and Munns 1984) supports, for this population, the supposition that diapause is an adaptation.

The timing of diapause did not differ in the two ponds simply because the generations matured at different times of year, with all copepods following the same diapause sequence regardless of pond type. Although first generation females in both ponds did make subitaneous eggs early in life and diapausing eggs later (in agreement with the bet-hedging strategy found to be adaptive in highly variable environments), second generation females made only diapausing eggs in Bullhead Pond while in Pond A they switched back to subitaneous eggs. The results of the reciprocal transfer experiment support the hypothesis that *D. sanguineus* populations are adapted to the conditions specific to the pond in which they reside. Females moved to a new pond continued to make diapausing eggs in accordance with the sequence followed in their pond of origin, rather than that followed by the native population of the new environment. We conclude that the copepods cannot sense the type of pond they are in and then respond appropriately. Rather, the conditions inducing diapause appear to be distinct for populations living in different pond types.

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References

Allan JD (1982) Life history variation in a freshwater copepod: evidence from population crosses. Am Soc Limnol Oceanogr Abstracts

Beck SD (1980) Insect photoperiodism. 2nd ed. Academic Press, New York

Bouligand Y (1972) Twisted fibrous arrangements in biological materials and cholesteric mesophases. Tissue Cell 4:189–217

Brewer RH (1964) The phenology of *Diaptomus stagnalis* (Copepoda: Calanoida): the development and the hatching of the egg stage. Physiol Zool 37:1–20

Champeau A (1970) Etude de la vie latente chez les Calanoides (Copépodes) Charactéristiques des eaux temporaires de Basse-Provence. Ann Fac Sci Marseille 44:155–189

Cooley JM (1971) The effect of temperature and light on the development of resting eggs of *Diaptomus oregonensis*. Limnol Oceanogr 16:921–926

Dingle H (1978) Evolution of insect migration and diapause. Springer, New York

Dingle H (1981) Geographic variation and behavioral flexibility in milkweed bug life histories. In: Denno RF, Dingle H (eds) Insect life history patterns. Springer, New York

Endler JA (1977) Geographic variation, speciation, and clines. Princeton Univ Press, Princeton

Flint APF, Renfree MB, Weir BJ (1981) Embryonic diapause in mammals. J Reprod Fert Suppl 29

Gharagozlou-Van Ginneken ID, Bouligand Y (1973) Ultrastructures tégumentaires chez un crustace copepode *Cletocamptus retrogressus*. Tissue Cell 5:413–439

Gillespie JH (1977) A general model to account for enzyme variation in natural populations. III multiple alleles. Evolution 31:85–90

Gillespie JH, Langley C (1976) Multilocus behavior in random environments. I random Levene models. Genetics 82:123–137

Hairston NG Jr., Walton WE, Li KT (1983) The causes and consequences of sex-specific mortality in a freshwater copepod. Limnol Oceanogr 28:935–947

Hairston NG Jr., Munns WR Jr (1984) The timing of copepod diapause as an evolutionarily stable strategy. Amer Natur (in press)

Harper JL (1977) Population biology of plants. Academic Press, New York

Hoy MA (1978) Variability in diapause attributes of insects and mites: some evolutionary and practical implications. In: Dingle H (ed) Evolution of insect migration and diapause. Springer, New York

Hutchinson GE (1967) A treatise on limnology, vol 2, Wiley, New York

Levins R (1968) Evolution in changing environments. Princeton Univ Press, Princeton

Löffler H (1964) Vogelzug and Crustaceenverbreitung. Zool Anz suppl 27:311–316

Marcus NH (1980) Photoperiodic control of diapause in the marine calanoid copepod *Labidocera aestiva*. Biol Bull 159:311–318

Marcus NH (1982) Photoperiodic and temperature regulation of diapause in *Labidocera aestiva* (Copepoda: Calanoida). Biol Bull 162:45–52

Mazur GD, Regier JC, Kafatos FC (1982) Order and defects in the silkmoth chorion, a biological analogue of a cholesteric liquid crystal. In: King, Akai (eds) Insect ultrastructure, vol 1, Plenum, New York

Mellors WK (1975) Selective predation of ephippial *Daphnia* and the resistance of ephippial eggs to digestion. Ecology 56:974–980

Showers WB (1981) Geographic variation of the diapause response in the European corn borer. In: Denno RF and Dingle H (eds) Insect life history patterns. Springer, New York

Thienemann A (1950) Verbreitungsgeschichte der Süßwassertierwelt Europas. Die Binnengewässer vol 18, Stuttgart

Walker TJ (1980) Mixed oviposition in individual females of *Gryllus firmus*: graded proportions of fast-developing and diapause eggs. Oecologia (Berlin) 47:291–298

Watras CJ (1980) Subitaneous and resting eggs of copepods: relative rates of clutch production by *Diaptomus leptopus*. Can J Fish Aquat Sci 37:1579–1581

Wilson MS, Yeatman HC (1959) Free-living Copepoda. In: Edmondson WT (ed) Fresh-water biology. John Wiley, New York

Wyngaard GA (1982) The adaptive significance of heritable life history variation in a freshwater copepod. Am Soc Limnol Oceanogr Abstracts

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