

Distributions of diploid and pentaploid brine shrimp *Artemia parthenogenetica* in an illuminated thermal gradient

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We explored the behavioral response to horizontal gradients of light and heat of sympatric diploid and pentaploid *Artemia parthenogenetica*. When exposed to a uniformly illuminated thermal gradient, pentaploids had a mean distribution that was oriented toward temperatures 2.9°C higher than that of diploid animals. In a light gradient, diploids were distributed at higher light intensities than pentaploids. When the distributions in an evenly illuminated thermal gradient were compared with those occurring when thermal and light gradients ran in opposite directions, it was found that diploids tended to occur at lower temperatures, whereas pentaploid distributions were not significantly different for the two conditions. Our findings suggest that pentaploids are not as strongly attracted to light as diploids, in either the presence or the absence of a thermal gradient. We predict that in the field, warmer areas of a pond may be exploited by pentaploids during the day and by diploids during the night.

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Nous avons étudié le comportement en présence de gradients horizontaux de lumière et de chaleur chez des *Artemia parthenogenetica* sympatriques diploïdes et pentaploïdes. Lors d'une exposition à un gradient thermique à lumière uniforme, les pentaploïdes se situaient sur le gradient à une température moyenne de 2,9°C plus élevée que les animaux diploïdes. Dans un gradient de lumière, les individus diploïdes se répartissaient à une intensité lumineuse plus grande que les pentaploïdes. En comparant la répartition des deux groupes dans un gradient thermique d'illumination égale à leur répartition dans des gradients opposés de température et de lumière, les individus diploïdes semblaient préférer les températures plus basses, alors que la répartition des pentaploïdes ne variait pas significativement en fonction des deux facteurs. Il semble donc que, contrairement aux diploïdes, les pentaploïdes ne soient pas fortement attirés vers la lumière, en présence ou en l'absence d'un gradient thermique. Ces résultats nous permettent de croire que, dans un étang, les zones chaudes sont probablement exploitées par les pentaploïdes durant le jour et par les diploïdes durant la nuit.

[Traduit par la réduction]

Introduction

Two factors that influence zooplankton migrations are light and temperature (Berzins 1958; Hutchison 1967; Vinogradov 1970; Forward 1976; Koslow 1979). The diurnal light cycle serves as a proximal cue for migration. It may also directly influence fitness by modifying exposure of the zooplankton to predators, such as fishes, that rely on vision for prey location and capture (Zaret 1980; O'Brien 1987). Similarly, since temperature usually varies with depth, vertical migration has the potential to influence fitness by altering temperature-dependent physiological processes (McLaren 1963, 1974; Orcutt and Porter 1983; see also review by Lampert 1989). Sympatric zooplankton species may differ in their vertical distributions because of disparate migratory behaviors (Berzins 1958). Distributional differences may also occur within genetically variable populations if the component individuals have different responses to light or temperature.

Brine shrimp of the genus *Artemia* occur in inland salt lakes and solar salt works where very high salinities exclude fish and other visual predators. *Artemia* tend to be highly variable in horizontal and vertical distribution (Lenz 1980; Conte and Conte 1988; Conte et al. 1988). Similarly, Dojmi Di Delupis and Rotondo (1988) found that the phototactic response of *Artemia franciscana* nauplii varied with density of nauplii and the intensity and spectral composition of the light. In a given experiment it was frequently observed that some individuals were photopositive, others were photonegative, and still others had an uncertain response to the light.

One obvious explanation for variability of this type is the presence of genetic variation in sexually reproducing species such as *A. franciscana*. *Artemia parthenogenetica* reproduces by obligate parthenogenesis and is notable for having sympatric

diploid and pentaploid individuals. Polyploidy, as a dramatic mutational event, may greatly alter the physiological characteristics of organisms (Levin 1983). For instance, polyploid animals are usually more tolerant of temperature change than sympatric diploids (Schultz 1982). Survival of pentaploid *Artemia* is higher than that of sympatric diploids at both high and low temperature extremes (Zhang and Lefcort 1991). Yet such measures of tolerance are not good predictors of behavior and, moreover, at the behavioral level very little is known about the effects of polyploidy. Zhang and Lefcort (1991) also presented diploid and polyploid *Artemia* with a thermal gradient in the absence of light; pentaploids were consistently recovered at lower mean temperatures than diploids.

In nature, vertical temperature gradients are usually associated with light gradients. We wondered if the combined stimuli of light and heat might differentially affect the behavior of diploids and pentaploids. We examine herein the distributions of diploid and pentaploid *A. parthenogenetica* in continuously variable horizontal gradients of light and temperature in the laboratory. Although shallow horizontal gradients differ from vertical gradients in several respects, they are useful in exploring the ability of individuals to distinguish and respond to variation in light and temperature (Forward 1986; Wollmuth et al. 1987; Sievert and Hutchison 1988).

Methods

Diploid and pentaploid *A. parthenogenetica* cysts were collected from the Dong Fang Hong saltern in northeastern China in July 1985 and hatched in our laboratory at Oregon State University. Three diploid clones and three pentaploid clones were isolated. These clones were maintained in natural seawater with a salinity of approximately 35 parts per thousand (ppt) under 24-h cool-white fluorescent lighting at an intensity of 4000–5000 lx. The temperature was maintained at 25 ±

0.5°C. The unicellular green alga *Dunaliella tertiolecta* was used as food. Experimental animals were maintained at low density in glass aquaria, and food was added daily to obtain a concentration of approximately 17 000 000 cells/mL.

Experiments were performed using equal numbers of 15-day-old juveniles from each of three diploid clones to obtain diploid mixtures. Pentaploid mixtures were obtained in a similar manner. All experiments were conducted in a grey epoxy-painted wooden container measuring 273.0 × 27.0 × 46.0 cm. The container was filled with natural seawater to a depth of 1.5 cm. The warm end of a thermal gradient was created by placing a 1-L beaker of water containing two 200-W immersion coils at one end of the container. The cold end was created by placing a 5-L glass jar filled with ice at the opposite end. Both the hot and cold sources were separated from the animals with fine plastic mesh screen placed 19 cm from the container ends. The length of the container available to the animals was therefore 234.0 cm. This length was further divided by faint pencil lines into 12 zones, each 19.5 cm long. Temperatures, to the nearest 0.1°C, were recorded at the center of each zone along the midline of the chamber. Temperature was recorded using a K-type thermocouple connected to an Omega HH 82 digital thermometer. No vertical stratification of temperature was observed. Temperatures in the gradient ranged from an average of 15.0°C in zone 1 to an average of 31.8°C in zone 12. Temperatures in each zone varied by less than 1.5°C between trials. The thermal gradient was not linear because temperatures changed more rapidly at the ends than in the middle of the experimental chamber (Fig. 1).

Five experiments were conducted, two with uniform illumination and three with light as a stimulus: (1) uniform illumination at a constant temperature; (2) uniform illumination in the presence of a thermal gradient; (3) a point source of light at a constant temperature; (4) a point source of light placed over the cold end of a thermal gradient; and (5) a point source of light over the warm end of a thermal gradient.

For the uniform-illumination experiments (expts. 1 and 2) two 236-cm cool-white fluorescent tubes were used; these produced an intensity of 525 lx at the water's surface. For the experiment with light as a stimulus at a constant temperature (expt. 3), two 58-cm cool fluorescent bulbs were placed across zone 12, creating a gradient ranging from 770 lx in zone 12 to 2 lx in zone 1. For experiment 4 the two lights were positioned over zone 1, whereas in experiment 5 the lights were placed over zone 12.

Once the gradient had been established, groups of four diploids and four pentaploids from the mixtures were added to each of the 12 zones. Zhang and Lefcort (1991) found no difference in thermal distributions when the two cytotypes were placed either separately or together in the container. They also found that the density of animals had no effect on thermal distributions. Preliminary results indicated that the distribution of the animals after 1 h in the presence of the gradient did not statistically vary from distributions after 2 and 4 h. Therefore, at the end of 1 h, barriers were placed between the zones, temperatures were measured, and the number of animals of each cytotypic present in each zone was recorded. All animals within a zone were assigned the same temperature. Tests were conducted at a constant air temperature of 21.1°C. For the uniform-illumination experiments nine trials of 48 diploids and 48 pentaploids were run for each experiment. For the experiment with light as a stimulus, five trials were run for each experiment. Each trial involved different animals.

Data for Figs. 2, 5, and 6 are smoothed for the purpose of illustration and are depicted in relation to the distributions of the animals in the absence of a thermal gradient (controls). The data were smoothed by the following calculation:

$$(X_c + \frac{1}{2}X_{c-1} + \frac{1}{2}X_{c+1})/2$$

where X_c is the data point to be smoothed and X_{c-1} and X_{c+1} are the data points immediately before and after X_c .

All mean temperatures reported are presented with standard error. All P values reported are from pooled t -tests unless otherwise noted. All χ^2 values reported are from heterogeneity χ^2 tests (Sokal and Rohlf 1969).

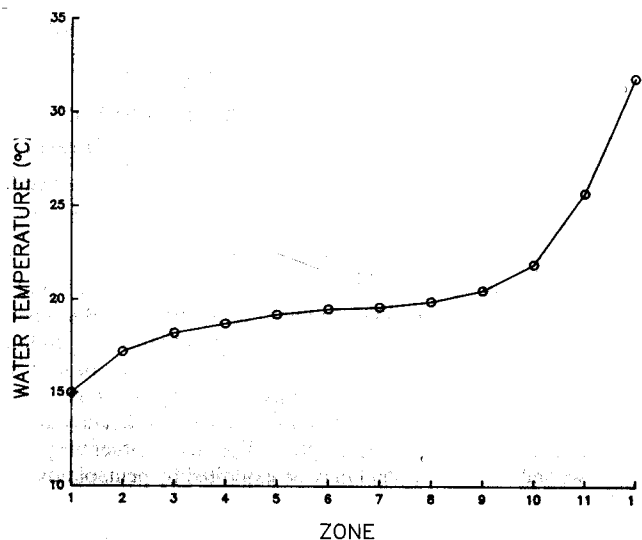


FIG. 1. Mean temperature in each zone of the thermal gradient.

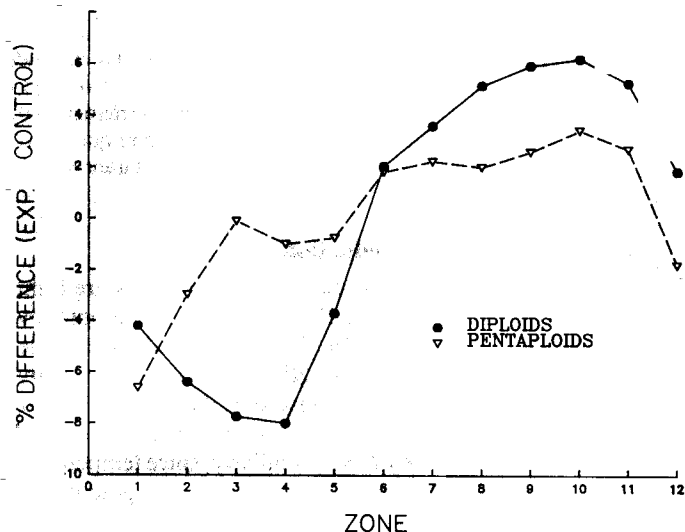


FIG. 2. Smoothed distribution of diploids and pentaploids in an evenly illuminated thermal gradient. The source of heat is at zone 12. The control is the distribution of the two cytotypes under uniform illumination in the absence of a thermal gradient.

Results

Uniform illumination

The distributions of the diploids and the pentaploids were significantly different in both the absence ($\chi^2 = 64.6$, $df = 11$, $P < 0.001$) and the presence ($\chi^2 = 29.8$, $P < 0.005$) of a thermal gradient (Table 1). The mean temperature taken over all cells and replicates of the animals under constant illumination of 525 lx was $21.1 \pm 0.2^\circ\text{C}$ for the diploids and $24.0 \pm 0.3^\circ\text{C}$ for the pentaploids. The mean temperature of the diploids was significantly different from that of the pentaploids ($P < 0.001$). The mean temperatures of each cytotypic in an evenly illuminated thermogradient were significantly lower for diploids and higher for pentaploids than the means found by Zhang and Lefcort (1991) in a dark thermogradient ($P < 0.001$ for both cytotypes; Fig. 2).

TABLE 1. Distributions of animals under uniform light in the absence or presence of a thermal gradient

Zone	Diploid			Pentaploid		
	No gradient (%) (n = 432)	With gradient (%) (n = 432)	Difference	No gradient (%) (n = 432)	With gradient (%) (n = 432)	Difference
1	4.2	2.2	-2.0	10.1	1.8	-8.3
2	10.5	2.1	-8.4	5.4	2.5	-2.9
3	9.6	2.9	-6.7	5.4	7.8	2.4
4	15.5	6.3	-9.2	6.0	3.7	-2.3
5	13.2	6.3	-6.9	6.3	4.5	-1.8
6	4.8	13.0	8.2	4.1	7.0	2.9
7	7.2	5.9	-1.3	2.5	5.8	3.3
8	5.1	13.9	8.8	7.6	7.0	-0.6
9	7.8	12.2	4.4	6.9	12.8	5.9
10	3.0	9.2	6.2	8.2	7.4	-0.8
11	12.0	20.1	8.1	8.8	18.2	9.4
12	7.1	5.9	-1.2	28.7	21.5	-7.2

NOTE: The heat source was at zone 12.

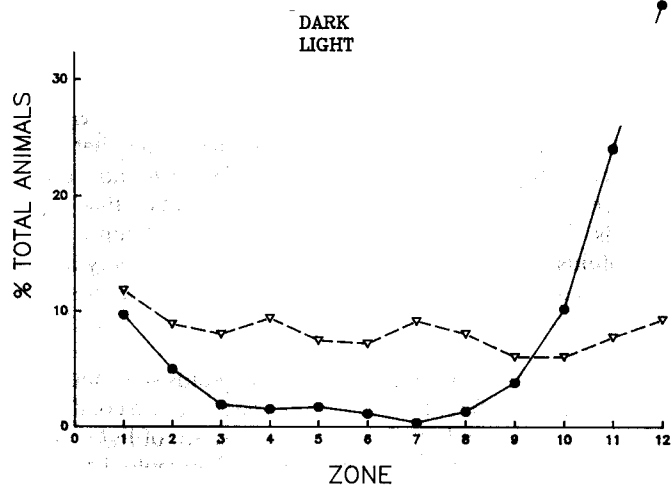


FIG. 3. Smoothed distribution of diploids in the absence of a thermal gradient both in the dark and with a point source of light over zone 12.

Light as a stimulus

When a point source of light was placed over zone 12 the distributions of the two cytotypes were significantly different in both the presence ($\chi^2 = 85.7$, $P < 0.001$) and the absence ($\chi^2 = 60.2$, $P < 0.001$) of a thermal gradient. Diploids appear to be strongly drawn to light (Fig. 3). The number of diploids exposed to a point source of light present in zones 10, 11, and 12 was greater than the number present when the container was not illuminated (paired t -test, $P = 0.03$). The number of pentaploids exposed to a point source of light present in zones 10, 11, and 12 was not significantly different from the number present in a dark container (paired t -test, $P = 0.241$). Pentaploids seem to be only weakly attracted to light (Fig. 4).

Both the diploids and the polyploids congregated in warm zones to the exclusion of brightly illuminated zones (Fig. 5). The mean temperature of diploids exposed to a point source of light was significantly lower than evenly illuminated diploids

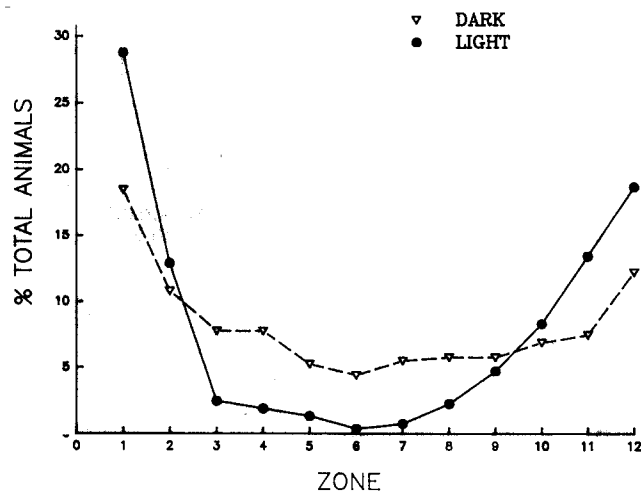


FIG. 4. Smoothed distribution of pentaploids in the absence of a thermal gradient both in the dark and with a point source of light over zone 12.

($P < 0.001$). The mean temperature of the pentaploids was not significantly different for the two conditions ($P = 0.35$).

When a point source of light is placed over the warm end of a thermal gradient the mean temperatures occupied by the two cytotypes were not significantly different ($P = 0.09$). Both diploids and pentaploids oriented toward the warm, bright zones (Fig. 6).

Discussion

Light is a stimulus that triggers migrations of parthenogenic *Daphnia* (Ringelberg 1964; McNaught and Hasler 1964) and other aquatic species (Vinogradov 1970; Forward 1987). In an aquatic habitat, different species may have different vertical distributions due to disparate migratory behavior. Within populations, individuals may show differential responses to

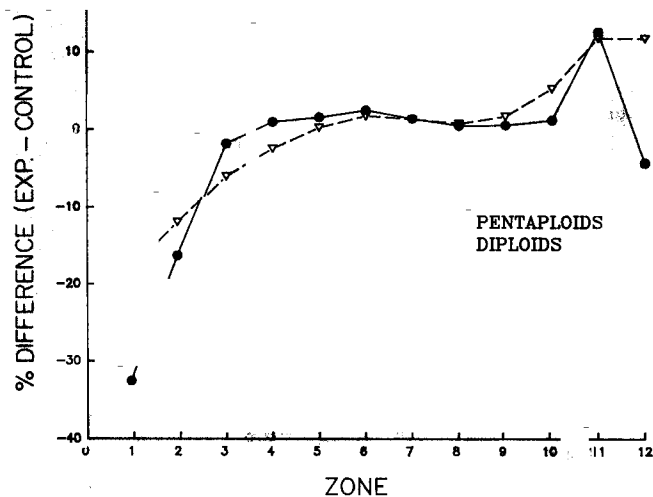


FIG. 5. Distribution of diploids and polyploids with a heat source at zone 12 and a point source of light over zone 1. The control is the distribution of the two cytotypes under uniform illumination in the absence of a thermal gradient.

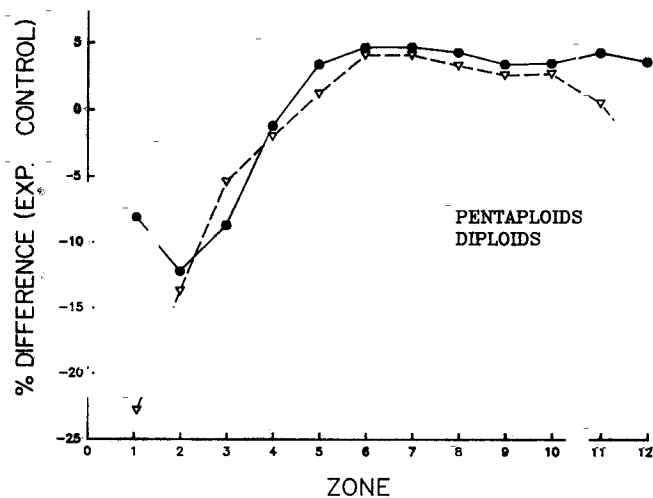


FIG. 6. Distribution of diploids and polyploids with both a heat source and a point source of light at zone 12. The control is the distribution of the two cytotypes under uniform illumination in the absence of a thermal gradient.

temperature and light intensity due to genetic effects such as chromosome doubling.

Polyploid plants have been found to be more tolerant of environmental stresses such as temperature extremes, poor soil quality, and pathogens than have diploids (Levin 1983). Similarly, pentaploid *Artemia* have higher survival rates at both extremely low (0°C) and high (37.5°C) temperatures (Zhang and Lefcort 1991). Because pentaploids are more robust, they may not be under strong selective pressure to be responsive to stimuli such as light and nonlethal extremes of temperature. This would afford pentaploid *Artemia* a high potential to exploit a wider range of temperature microhabitats, both spatially and temporally.

Our results suggest that in the laboratory, the temperatures at which the two cytotypes were found depends on the presence or absence of light. The mean temperature of the diploids was

23.9 ± 0.4°C in a dark thermal gradient (Zhang and Lefcort 1991) and 21.1 ± 0.2°C in an evenly illuminated thermal gradient. The mean temperature of the pentaploids was 22.2 ± 0.4°C in the dark (Zhang and Lefcort 1991) and 24.0 ± 0.3°C in an illuminated thermal gradient.

During the day in the Dong Fang Hong salterns, shallow layers of illuminated water are likely to occur in close proximity to layers of extremely hot (>40°C) water (L. Zhang, personal observation). Polyploids, with their greater ability to survive at higher temperatures (Zhang and Lefcort 1991), would be better able to survive such encounters with hot water.

The ecological significance of the different thermal distributions of the two cytotypes remains to be determined by field studies. Although other ecological and physical factors besides light and temperature affect the vertical migration of *Artemia*, our laboratory studies suggest possible field behaviors. We predict that in the field, warmer areas of a pond may be exploited by pentaploids during the day and by diploids during the night.

Pentaploid animals as a group exhibited less uniformity than diploids in their location along the thermal gradient. After the thermal gradient was established under even illumination (Fig. 2), a greater proportion of diploid than polyploid animals altered their locations to move to warm-water zones. The modest reaction of the polyploids to the presence of the thermal gradient suggests that they are less responsive to environmental stimuli. The difference in distribution between experimental animals and controls is greater for diploids than pentaploids, indicating a greater response of diploids to environmental stimuli. Under constant illumination the variance of the selected temperatures within trials of the pentaploids was more than twice that of the diploids. Similarly, Zhang and Lefcort (1991) found that the variance of pentaploids was one and a half times that of the diploids. This could also be due to the strong preference of the pentaploids for the ends of the container in both evenly illuminated and dark conditions in the absence of a thermal gradient. Although the evenly illuminated diploids were found at a significantly different mean temperature than the pentaploids exposed to a point source of light, the pentaploids were not found at significantly different temperatures under the two conditions. This suggests that only the presence or absence of light, not its intensity, affects the thermal distributions of pentaploid *Artemia*.

Our results suggest that pentaploids are not as strongly attracted to light as diploids, in either the presence or absence of a thermal gradient. Although light stimuli may trigger and direct the migrations of *Artemia*, thermal gradients may also strongly influence such migrations. This has potential consequences for the survival of *Artemia* in solar salt works, where lethal afternoon temperatures are common in the surface layer of the ponds. *Artemia* may have been selected to develop overriding thermoregulatory behavior, thus avoiding being drawn to lethal-temperature layers by light stimuli.

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