

Photoperiodic response of sexual reproduction in the *Daphnia pulex* group is reversed in two distinct habitats

Abstract—Two traditional species in the *Daphnia pulex* group (*D. pulex* and *D. pulicaria*) are not completely reproductively isolated. The microhabitats they dominate are distinct but overlap geographically, and migration between the microhabitats is not uncommon. Their species identity is not fully supported by a recent molecular study. A laboratory experiment shows that *Daphnia* clones isolated from two populations of each species switch to sexual reproduction in response to totally different photoperiodic cues, so that their mating seasons are expected to differ. This phenomenon is most likely due to local adaptation of the two species to their respective distinct microhabitats. The phenomenon, together with the peculiarity of the study system, is interpreted as an ongoing allochronic speciation process in aquatic systems.

Much has been debated about the origin of species, and the mode of sympatric speciation is particularly contentious (White 1978; Bush 1994). Allochronic speciation is a special form of sympatric speciation, in which differences in mating season contribute to the divergence of two taxa that may interbreed otherwise (White 1978). In animals, there are a few cases in which allochronic speciation is thought to occur, all of which are from terrestrial systems (White 1978). Here I report the results from an experiment with the freshwater microcrustacean *Daphnia* and interpret the results in light of the possibility of allochronic speciation in this aquatic system.

The *Daphnia pulex* group consists of obligately asexual and cyclically parthenogenetic populations. Cyclically parthenogenetic populations normally reproduce asexually during the growing season in benign environments and produce diapausing eggs (ephippia) sexually prior to the arrival of seasonally harsh periods (e.g. drought in summer in temporary ponds and adverse winters in permanent lakes). Seasonal timing of sexual reproduction is important in determining an individual's fitness (Carvalho and Hughes 1983; Hobæk and Larsson 1990), which is more or less synchronized in a particular season within populations (Stross 1987; Threlkeld 1987) and has a genetic basis (Carvalho and Hughes 1983; Yampolsky 1992; Deng 1996). Two traditional species within the *D. pulex* group (*D. pulex* and *D. pulicaria*) normally live in distinct but geographically overlapping habitats. *D. pulex* occupies temporary ponds and *D. pulicaria* inhabits permanent lakes. The two species are morphologically similar (Brandlova et al. 1972) but are fixed for alternative alleles at the diagnostic lactate dehydrogenase (LDH) locus (Hebert et al. 1988, 1989; Lynch et al. 1989). Mating is normally random within cyclically parthenogenetic populations of the two species (Lynch and Spitze 1994; Lynch and Deng 1994; Deng and Lynch 1996). Interbreeding between the two species is not uncommon in nature; hybrids are viable and fertile (Hebert 1987; Hebert and Crease 1980, 1983) but normally reproduce purely asexually (Crease et al. 1990). Genetic introgression between the two species is rare (Cerny and

Hebert 1993). A recent molecular phylogenetic study does not support the conclusion that these two traditional taxa are distinct species (Lehman et al. 1995).

Populations of traditional *D. pulex* and *D. pulicaria* are widely distributed in midwest Oregon. Their habitats overlap geographically and have distinct phenologies. The temporary ponds inhabited by *D. pulex* usually begin to fill with water by spring and dry up in summer, whereas the populations of *D. pulicaria* in permanent habitats (lakes and reservoirs) usually decline in autumn. Co-occurrence of the two species in a single pond or lake has not yet been found in midwest Oregon, although it has been reported elsewhere (Cerny and Hebert 1993). To investigate the patterns of sexual reproduction in different seasons, I examined four populations in midwest Oregon under different photoperiods. I found that sexual reproduction was induced in *D. pulicaria* from permanent habitats by short-day photoperiods and in *D. pulex* from temporary ponds by long-day photoperiods. The opposite photoperiodic responses are adaptive with regard to the respective dominant habitats of the two species. The phenomenon should promote sexual reproduction in different seasons and serve as a premating isolating mechanism, thus reducing gene flow between the two species.

All four experimental populations are within ~250 km of each other. Two populations of *D. pulicaria* were collected from permanent habitats (Little Cultus Lake in the Oregon Cascades and Dorena Reservoir in the Willamete Valley). Two *D. pulex* populations were collected from temporary ponds on the Oregon Coast (Haceta Pond and Florence Dune Pond). According to the traditional criteria (Brooks 1957; Hebert et al. 1988, 1989; Lynch et al. 1989), the two permanent populations were classified as *D. pulicaria* and the two temporary pond populations as *D. pulex*. Consistency with Hardy-Weinberg proportions at the polymorphic loci phosphoglucosmutase (PGM) and phosphoglucosomerase (PGI) revealed that these four populations are cyclically parthenogenetic and mating is effectively random within each population (Deng unpubl.). Dozens of random individuals from each population were isolated into small individual beakers containing about 200 ml of water from Dorena Reservoir. The isolated individuals were fed with the green alga *Scenedesmus* and kept at 15°C and 12:12 L/D photoperiod. All isolates reproduced asexually in the laboratory and formed clonal cultures.

Twenty clones from each of the four populations were used in the following experiment. I assumed that a day consisted of the hours between sunrise and sunset and chose four photoperiods (10, 13.5, 15.5, 17 h of light d⁻¹) to cover all seasons experienced in nature at 42–46°N. Within each photoperiod, each clone of each population was represented by two replicates. Each replicate was initiated by one immature individual from its clonal culture as the first generation; two newborns from the first clutch of the first generation were used to start

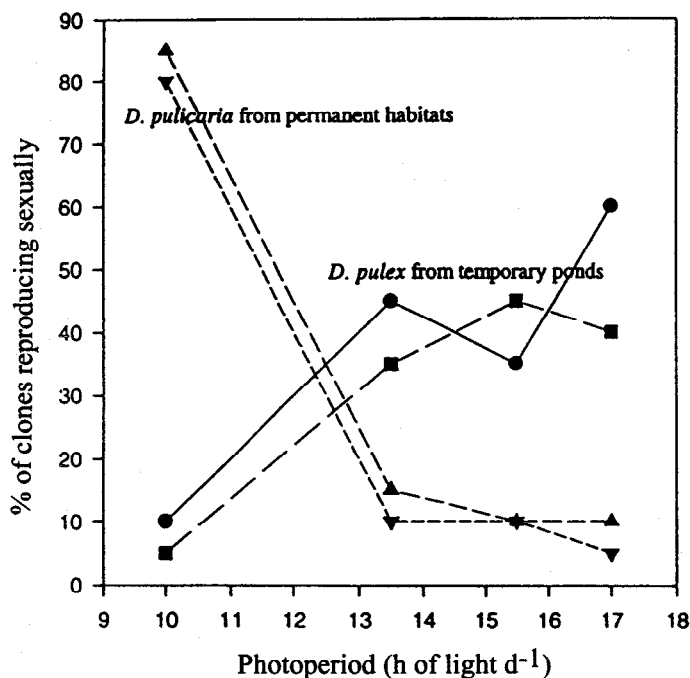


Fig. 1. Percentage of clones reproducing sexually (i.e. having produced at least one ephippia) in the four experimental photoperiods. Data shown for Dorena (▲) and Little Cultus (▼) *Daphnia pulicaria* populations and for the Florence Dune (●) and Haceta (■) *Daphnia pulex* populations.

the second generation; then four newborns from the second clutch of the second generation were used to start the third generation. The experimental temperature was 10°C and the culture conditions were 120 ml of water with 300,000 cells of *Scenedesmus* ml⁻¹ in a beaker. The water was taken from the Dorena Reservoir, aged for at least 1 month, and filtered before use. The culture water was changed every 4 d.

The first two generations served to acclimatize the clones to the standard experimental conditions. In the third generation, all replicates were monitored until the 20th day after maturity, with maturity judged by the first appearance of sexual or asexual reproduction occurring in the beaker. During the 20 d after maturity, the number of released ephippia was counted for each replicate of each clone, and the released asexual offspring were discarded every 4 d when changing culture water. During the experimental period (20 d starting at maturity), each individual reproduced about three ephippia, or five asexual clutches, or some combination of them. Under a given photoperiod, if any ephippium was produced in either replicate of a clone, then that clone was classified as having reproduced sexually. The genetic diversity of the future populations hatched from diapausing eggs depends highly on the proportion of clones engaging in sexual reproduction. Hence, the proportions of clones reproducing sexually in each photoperiod were computed for each population (Fig. 1). The mean number of ephippia produced per individual in each photoperiod is reported in Table 1 to indicate the intensity of sexual reproduction (Deng 1996).

Sexual reproduction was induced in *D. pulicaria* by short days and asexual reproduction was maintained in long days (Fig. 1). In *D. pulex*, short days maintained asexual reproduc-

Table 1. Mean number of ephippia produced per individual in each photoperiod for the four study populations. The numbers in parentheses are 1 SE (calculated from replicates of all clones in each photoperiod). Each reported value is based on the data from 160 individuals (for each population in a given photoperiod, there were 20 clones, each with two replicates, and each replicate contained four individuals).

| Photoperiod (h of light d ⁻¹) | <i>Daphnia pulex</i> from temporary ponds | | <i>Daphnia pulicaria</i> from permanent lakes | |
|---|---|----------------|---|----------------|
| | Florence Dune | Haceta | Dorena | Little Cultus |
| 10.0 | 0.04 (0.00) | 0.03 (0.00) | 2.22 (0.07) | 2.01 (0.07) |
| 13.5 | 1.26 (0.04) | 1.05 (0.03) | 0.56 (0.02) | 0.48 (0.02) |
| 15.5 | 1.19 (0.05) | 1.42 (0.07) | 0.05 (0.01) | 0.06 (0.00) |
| 17.0 | 1.32 (0.05) | 1.49 (0.06) | 0.04 (0.00) | 0.02 (0.00) |

tion while longer photoperiods induced progressively greater sexual reproduction (Fig. 1). The above pattern, observed for the proportion of sexual clones in different photoperiods was corroborated by the data on the intensity of sexual reproduction, as quantified by the mean number of ephippia produced per individual in different photoperiods (Table 1).

The threshold pattern for the two permanent habitat *D. pulicaria* populations corroborates previous studies of photoperiodic response of sexual reproduction in *Daphnia* (cf. Stross and Hill 1965, 1968; Carvalho and Hughes 1983; Hobaek and Larsson 1990). The reversed pattern of photoperiodic response of sexual reproduction, as observed for the two temporary habitat *D. pulex* populations, has not been documented previously for *Daphnia*.

Because of the large scale of the experiment (640 beakers involved), data on the production of male offspring were not recorded. Recent observations on one *D. pulex* population from a temporary pond (H.-W. Deng and M. Pfrender unpubl.) indicate that male and ephippial egg production in different photoperiods are correlated, and the threshold pattern of the photoperiodic response of sexual reproduction as quantified by the production of ephippia or males is similar. This result is consistent with previous studies in the laboratory or in situ (Stross 1987; Threlkeld 1987).

Sexual reproduction in *Daphnia* is mainly determined environmentally, and photoperiod has been shown to be a key environmental cue for initiation and intensity of sexual reproduction (Stross 1987; Deng 1996). The observed threshold patterns of photoperiodic response of sexual reproduction imply that sexual reproduction occurs at different seasons for *D. pulicaria* and *D. pulex* in their respective habitats. *D. pulicaria* populations in permanent habitats normally reproduce sexually in late summer or early fall because of the onset of winter, whereas *D. pulex* populations in ephemeral habitats reproduce sexually in late spring or early summer because of summer drought (Threlkeld 1987). The four studied populations are not exceptional to the normal pattern in this aspect. The two permanent lake *D. pulicaria* populations reproduce sexually in fall, while the two temporary pond *D. pulex* populations reproduce

