# Ecological genetics of *Daphnia pulex* in a temporary prairie woodland pond

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## Introduction

Populations of *Daphnia pulex* in North America reproduce via cyclic or obligate parthenogenesis and the breeding system varies geographically. Obligate parthenogenesis occurs more commonly in eastern Canada, and cyclic parthenogenesis is more frequent in western Canada, with an abrupt transition coincident with the prairie/forest ecotone (Hebert *et al.* 1993). Cyclic parthenogens inhabiting intermittent habitats reproduce asexually by parthenogenesis under favourable environmental conditions. When conditions deteriorate, they switch to sexual reproduction to produce ephippial eggs capable of diapause for months to years (Fryer 1996). These cyclic parthenogens are usually in Hardy-Weinberg equilibrium at most gene loci and have high genotypic diversity (Innes *et al.* 1986). Multiple clones coexist in the same habitat (Hebert and Crease 1980), although spatial and temporal partitioning of the habitat with respect to clone frequency has been documented (Weider 1985, Weider and Hebert 1987).

Mean caparace length, brood size, lipid and hemoglobin concentration in the hemolymph vary seasonally in populations of *Daphnia pulex* (LaBerge and Hann 1990). The adaptive response observed may be either genotypic or phenotypic. Specific clones which produce more hemoglobin may increase in abundance under particular environmental conditions, or there may be an increase in production of hemoglobin by all individuals in the population in response to a change in environmental conditions. Hemoglobin concentrations have been shown to display an inverse correlation with oxygen concentration in *Daphnia pulex* (Hoshi and Kobayashi 1972).

The objectives of this study were: (1) to determine whether *D. pulex* reproduces via obligate or cyclic parthenogenesis, (2) to quantify the condition (brood size, lipid-ovary index) and hemoglobin concentration in the hemolymph of individuals in the population over the season, (3) to relate the occurrence of clones and clonal diversity with environmental conditions, and (4) to assess the genotypic and phenotypic components of the response of *D. pulex* to hypoxic conditions in an ephemeral woodland pond.

## Methods and Materials

Samples of *D. pulex* were collected weekly from May - July 1989 from Mist Net Pond, a shallow temporary woodland pond located on the beach ridge at the University of Manitoba Field Station (Delta Marsh). The pond fills with melt water in spring and is replenished with rainwater. Profiles of temperature and oxygen concentration were determined at 10 cm intervals through the water column using a YSI combination probe.

In the laboratory, individual adult females were transferred to 100 mL plastic cups containing artificial pond water (48 mg/L Na bicarbonate, 38 mg/L hydrated calcium sulphate, 30 mg/L $MgSO_4$, 0.5 mg/L KCl, Hebert and Crease 1980), and were fed every second day with 10 mL of a suspension of cultured algae, primarily *Scenedesmus*. At least 80 clones were established on each sampling date. Cups were kept at $18 \pm 2^{o}C$ in a controlled environmental chamber with a 14-hour photoperiod.

Carapace length was measured from the centre of the compound eye to the base of the carapace spine. Condition indices were determined as follows: egg stage (I - IV, according to Threlkeld 1979, modified as in LaBerge and Hann 1990), egg number, lipid-ovary index (0 - 3, according to Tessier and Goulden 1982), and haemoglobin concentration (Fox 1948, methods modified as in LaBerge and Hann 1990).

Cellulose acetate gel electrophoresis was used to determine multilocus genotypes (MLG) for clones of *D. pulex* using established methods (Hebert and Beaton 1993). Six polymorphic loci were examined: phosphoglucose isomerase (PGI), aldehyde oxidase (AO), glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH).

The breeding system was diagnosed using three criteria (Hebert *et al.* 1988) derived from the genotypic structure of the population: (1) the number of MLGs

identified from allozyme data, (2) expected probability of occurrence of the observed genotypic array generated by comparing genotypic frequencies at single loci with H-W expectations, and (3) observed genotypic or clonal diversity calculated as $G_0$ (Stoddart 1983). Values range from 1, where one genotype is present, to a maximum where all genotypes are present in equal numbers.

## Results

### *Environmental conditions*

Water temperature and dissolved oxygen concentration (ppm) decreased with depth in the water column on each sampling date (Table 1). However, there was no consistent pattern from May through July, in part due to the increasing thickness of the covering of duckweed (*Lemna minor)* on the entire water surface. Pond water depth declined from >60 cm in early May to <30 cm in late July. Depth increased by >10 cm temporarily in mid-June as a consequence of heavy rainfall on 12 June.

Dissolved oxygen concentration (ppm) was very low even at 10 cm depth throughout the season. Percent oxygen saturation (Fig. 1) attained its highest value (27.5%) soon after the heavy rainfall in June.

Table 1. Water temperature (T - °C), dissolved oxygen ($O_2$ - ppm), and percentage oxygen saturation (%) with depth (D - cm) in Mist Net Pond, Delta Marsh.

| D | T | $O_2$ | % | T | $O_2$ | % | T | $O_2$ | % |
|---|---|---|---|---|---|---|---|---|---|
| | 18 May | | | 24 May | | | 31 May | | |
| 10 | 15.0 | 1.2 | 12.3 | 12.9 | 1.3 | 12.7 | 13.0 | 2.0 | 19.6 |
| 20 | 14.0 | 1.0 | 10.0 | 12.5 | 0.9 | 8.7 | 12.0 | 1.9 | 18.2 |
| 30 | 13.9 | 1.0 | 9.5 | 12.5 | 0.8 | 7.8 | 12.0 | 1.8 | 17.3 |
| 40 | 13.6 | 0.9 | 9.0 | 12.0 | 0.7 | 6.7 | 12.0 | 0.8 | 7.7 |
| 50 | 12.8 | 0.9 | 8.8 | | | | | | |
| | 7 June | | | 14 June | | | 21 June | | |
| 10 | 12.3 | 1.9 | 18.3 | 13.0 | 2.8 | 27.5 | 17.5 | 2.2 | 23.7 |
| 20 | 12.1 | 1.8 | 17.3 | 12.5 | 1.9 | 18.4 | 17.0 | 2.0 | 21.3 |
| 30 | 12.1 | 1.3 | 12.5 | 12.5 | 1.0 | 9.7 | 17.0 | 1.8 | 19.2 |
| 40 | 12.1 | 1.2 | 11.5 | 12.7 | 0.9 | 8.8 | 15.5 | 1.2 | 12.4 |
| 50 | | | | 12.7 | 1.0 | 7.8 | | | |
| 60 | | | | 12.5 | 0.7 | 6.8 | | | |
| | 28 June | | | 5 July | | | 11 July | | |
| 10 | 15.0 | 1.0 | 10.3 | 20.0 | 1.1 | 12.4 | 17.0 | 1.6 | 17.1 |
| 20 | 14.8 | 0.7 | 7.1 | 19.5 | 0.9 | 10.1 | 17.0 | 1.3 | 13.9 |
| 30 | 14.5 | 0.6 | 6.1 | 18.0 | 0.8 | 8.7 | 17.0 | 1.1 | 11.7 |
| 40 | 14.3 | 0.6 | 6.1 | | | | | | |
| 50 | 14.3 | 0.7 | 7.1 | | | | | | |
| 60 | 14.0 | 0.6 | 6.0 | | | | | | |
| | 19 July | | | | | | | | |
| 10 | 19.0 | 1.1 | 12.2 | | | | | | |
| 20 | 18.2 | 0.9 | 10.1 | | | | | | |

### *Carapace length and brood size*

Brood sizes increased with carapace length on each sampling date (Fig. 2). Mean carapace length, overall size range, and mean brood sizes decreased (largely as a consequence of smaller numbers of large individuals) during the course of the study (Table 2).

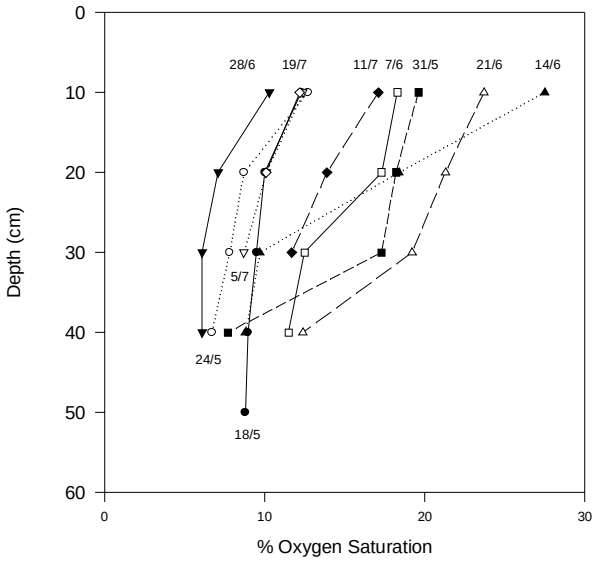


Figure 1. Oxygen saturation (%) with depth over the season in Mist Net Pond.

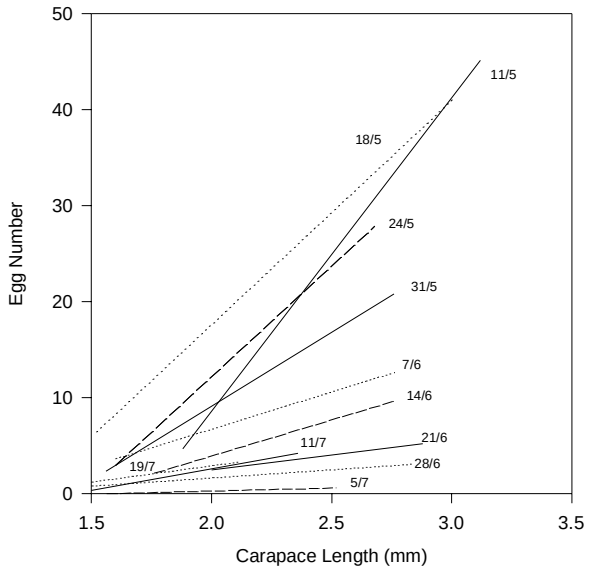


Figure 2. Seasonal change in the relationship between brood size and carapace length in parthenogenetic females of *D. pulex.*

Table 2. Summary of fecundity data for mature females of *D. pulex* in Mist Net Pond in 1989.

| Sample date | N | Mean Length X (mm) | Mean Egg Number Y | Regression Equation | r |
|---|---|---|---|---|---|
| 11 May | 65 | 2.52 | 25.7 | Y = 32.6X - 56.7 | 0.557 |
| 18 May | 70 | 2.20 | 22.3 | Y = 23.3X - 29.1 | 0.378 |
| 24 May | 75 | 2.09 | 14.1 | Y = 23.0X - 33.9 | 0.545 |
| 31 May | 79 | 2.15 | 11.4 | Y = 15.4X - 21.6 | 0.567 |
| 7 June | 80 | 2.22 | 8.4 | Y = 7.8X - 8.8 | 0.598 |
| 14 June | 80 | 2.25 | 5.8 | Y = 7.5X - 11.1 | 0.570 |
| 21 June | 80 | 2.38 | 3.6 | Y = 3.1X - 3.7 | 0.269 |
| 28 June | 80 | 2.05 | 1.7 | Y = 1.7X - 1.8 | 0.286 |
| 5 July | 80 | 1.93 | 0.2 | Y = 0.6X - 1.0 | 0.150 |
| 11 July | 80 | 1.83 | 1.8 | Y = 4.5X - 6.4 | 0.465 |
| 19 July | 60 | 1.66 | 1.7 | Y = 3.4X - 3.9 | 0.407 |

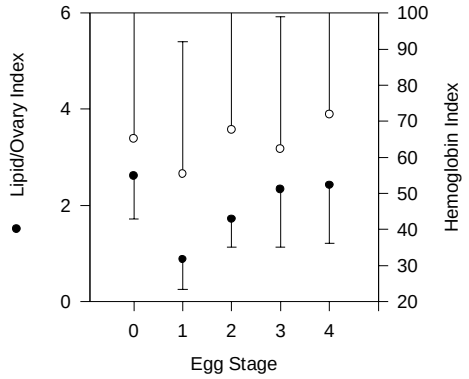


Figure 3. Relationship between lipid/ovary index (●) and egg stage, and hemoglobin index (❍) and egg stage for parthenogenetic females of *D. pulex.*

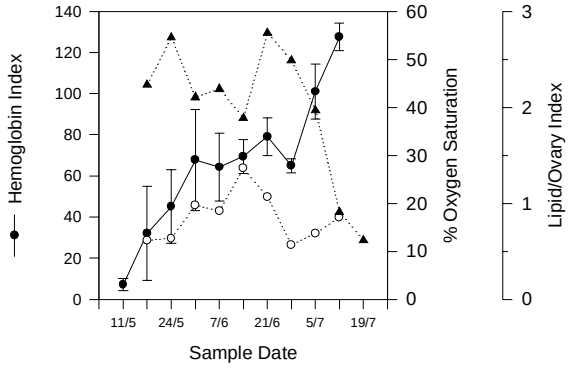


Figure 4. Mean hemoglobin index (●), oxygen saturation (❍), and lipid/ovary index (▲) over time in Mist Net Pond.

### *Lipid-ovary Index*

Lipid-ovary index, standardized to egg stage (Fig. 3), was highest when no eggs were present in the brood chamber (i.e. stage 0, when ovaries are enlarged and lipid concentrations are high). Once eggs had been released into the brood chamber (stage 1), the lipid-ovary index was at a minimum, significantly lower than in any other egg stage (ANOVA, $p<0.05$). Ovary development and lipid concentration increased over stages 2-4. There were no significant differences in values of the index among stages 0, 2-4 (ANOVA, $p>0.05$).

Lipid-ovary index values at each egg stage were significantly lower (ANOVA, $p<0.05$) on 5 July and 11 July than on all other dates in May and June (Fig. 4).

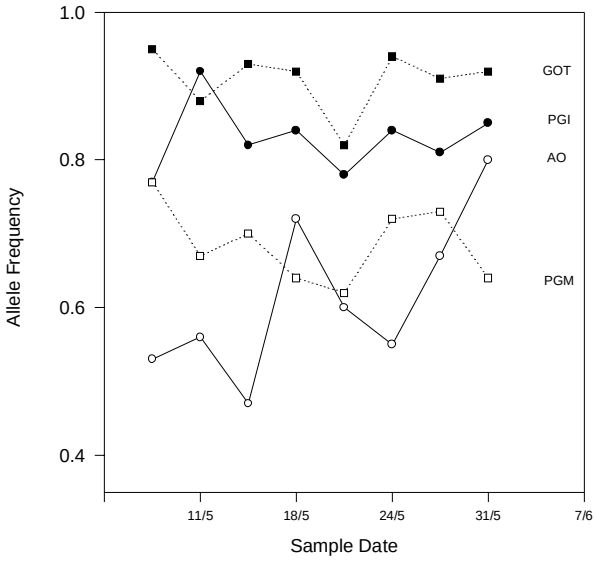


Figure 5. Allele frequencies for glutamine oxaloacetate transaminase (GOT), phosphoglucose isomerase (PGI), aldehyde oxidase (AO), and phosphoglucomutase (PGM) in populations of *D. pulex.*

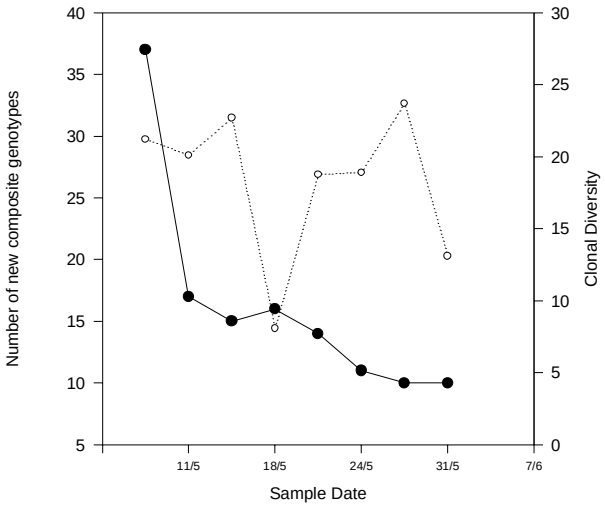


Figure 6. Number of new composite genotypes (●) and clonal diversity (❍) in populations of *D. pulex* in Mist Net Pond.

### *Haemoglobin*

Hemoglobin concentration in the hemolymph, standardized against egg stage (Fig. 3) was not significantly different among egg stages (ANOVA, $p>0.05$). However, mean hemoglobin concentration, measured by the hemoglobin index and averaged across all egg stages (Fig. 4), increased throughout the season. There was a weak correlation between hemoglobin concentration and water temperature (Pearson correlation, $r = 0.61$, $p = 0.08$), but showed no relationship with percentage oxygen saturation (Pearson correlation, $r = 0.2$, $p > 0.05$).

### *Gene frequencies and genotypes*

Four gene loci (PGI, AO, GOT, PGM) showed variation in allele frequencies (Fig. 5) over time. There were no significant differences in allele frequencies at each of the 4 gene loci over sampling dates (ANOVA, $p>0.05$). Allele frequencies were in Hardy-Weinberg equilibrium at each locus throughout the study. MDH and LDH patterns were very difficult to score consistently on the gels, so the data were not used in evaluating multilocus genotypes.

A total of 138 unique multilocus genotypes were identified among 609 individuals examined during the study. Frequency of appearance of new genotypes averaged 10-15 new clones on each sampling date after the initial date (Fig. 6). Two clones were more abundant than all others throughout the season (Table 3), although they comprised a very small percentage of the total number of clones in the pond on any given date. One of those two clones comprised ~30% of the population of ephippial females and males.

Table 3. Numbers of most abundant clones in MNP in 1989.

| Date | N | Clone 4 | Clone 5 | Clone 11 | Clone 13 |
|---|---|---|---|---|---|
| 11 May | 65 | 7 | 6 | 1 | 1 |
| 18 May | 70 | 3 | 0 | 2 | 5 |
| 24 May | 75 | 7 | 1 | 2 | 2 |
| 31 May | 79 | 14 | 3 | 0 | 2 |
| 7 June | 80 | 6 | 4 | 0 | 0 |
| 14 June | 80 | 9 | 7 | 0 | 4 |
| 21 June | 80 | 7 | 4 | 3 | 0 |
| 28 June | 80 | 14 | 7 | 5 | 0 |

Clonal diversity values, $G_0$, averaged 18.3 (8.08 - 23.7) (Fig. 6).

There were no significant relationships between the abundance of any clone and percentage oxygen saturation (at 10 cm depth) over time (Pearson product-moment correlation, $p>0.05$).

## Discussion

Intense microgradients of temperature, dissolved oxygen, light, nutrients, and pH have been recorded in waterbodies where a thick *Lemna* cover has developed (references in Goldsborough and Robinson 1985, Lewis and Bender 1961, Morris and Barker 1977). The *Lemna* cover reduces the effects of solar irradiance, maintaining low water temperatures despite elevated air temperatures, and also reducing diffusion of oxygen across the air-water interface. The extreme light attenuation caused by the *Lemna* cover (Goldsborough 1986) probably severely inhibited growth of phytoplankton in the pond, although phytoplankton biomass was not determined in this study.

The rapid decline in mean carapace lengths and brood sizes of adult females of *D. pulex* paralleled the development of the *Lemna* cover on the pond, and possibly reflects decreased availability of phytoplankton food. The lipid-ovary index for each egg stage varied little over time until July when anomalously low values were reported. This evidence substantiates the probable starvation of the adult females, resulting in reduced energy stores, limited moulting and growth, and very small brood sizes.

In Mist Net Pond, number of genotypes and clonal diversity were high, and Hardy-Weinberg equilibrium existed at all gene loci, suggesting that *Daphnia pulex* reproduces by cyclic parthenogenesis. Cyclically parthenogenetic populations of *D. pulex* have been previously documented in western Canada (Hebert *et al.* 1993) and midwestern United States (Lynch 1983, Innes *et al*. 1986). In the absence of a clear

environmental gradient, genotypic or phenotypic variation cannot be correlated with any environmental variable.

**Acknowledgments**

I thank Adrian Hawaleshka for assistance with sample collection and laboratory analyses. The study was funded in part by an NSERC Operating grant to BJH and an NSERC Summer Undergraduate Research Scholarship to AH.

**References**

Dave, G. 1984. Effects of waterborne iron on growth, reproduction, survival and haemoglobin in *Daphnia magna*. Comp. Biochem. Physiol. 78C: 433-438.

Fox, H.M. 1948. The haemoglobin of *Daphnia.* Proc. Roy. Soc. B135: 195-212.

Fryer, G. 1996. Diapause, a potent force in the evolution of freshwater curstaceans. Hydrobiologia 320: 1-14.

Goldsborough, L.G. 1986. Light penetration of a thick duckweed mat in a sheltered marsh channel. University Field Station (Delta Marsh) Annual Report 21: 46-58.

Goldsborough, L.G. and Robinson, G.G.C. 1985. Seasonal succession of diatom epiphyton on dense mats of *Lemna minor*. Can. J. Bot. 63: 2332-2339.

Hebert, P.D.N. and Beaton, M.J. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. Helena Laboratories, Beaumont, Texas.

Hebert, P.D.N., Ward, R.D. and Weider, L.J. 1988. Clonal-diversity patterns and breeding-system variation in *Daphnia pulex*, an asexual-sexual complex. Evolution 42: 147-159.

Hebert, P.D.N., Schwartz, S.S., Ward, R.D. and Finston, T.L. 1993. Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group. I. Breeding systems of Canadian populations. Heredity 70: 148-161.

Hoshi, T. and Kobayashi, K. 1972. Studies on physiology and ecology of plankton. XXVI. Promotion of haemoglobin synthesis by iron in *Daphnia magna* cultured under low oxygen conditions. Sci. Rep. Niigata Univ., Ser. D (Biology) 9: 55-62.

Innes, D.J., Schwartz, S.S. and Hebert, P.D.N. 1986. Genotypic diversity and variation in mode of reproduction among populations in the *Daphnia pulex* group. Heredity 57: 345-355.

LaBerge, S. and Hann, B.J. 1990. Acute temperature and oxygen stress among genotypes of *Daphnia pulex* and *Simocephalus vetulus* (Cladocera, Daphniidae) in relation to environmental conditions. Can. J. Zool. 68: 2257-2263.

Lewis, W.M. and Bender, M. 1961. Effect of a cover of duckweeds and the alga *Pithophora* upon the dissolved oxygen and free carbon dioxide of small ponds. Ecology 42: 602-603.

Lynch, M. 1983. Ecological genetics of *Daphnia pulex*. Evolution 37: 358-374.

Morris, P.F. and Barker, W.G. 1977. Oxygen transmission rates through mats of *Lemna minor* and *Wolffia* sp. and oxygen tension within and below the mat. Can. J. Bot. 55: 1926-1932.

Stoddart, J.A. 1983. A genotypic diversity measure. J. Heredity 74: 489-490.

Tessier, A.J. and Goulden, C.E. 1982. Estimating food limitation in cladoceran populations. Limnol. Oceanogr. 27: 707-717.

Threlkeld, S.T. 1979. Estimating cladoceran birth rates: the importance of egg mortality and the egg age distribution. Limnol. Oceanogr. 24: 601-612.

Weider, L.J. and Lampert, W. 1985. Differential responses of *Daphnia* genotypes to oxygen stress, respiration rates, hemoglobin content and low-oxygen tolerance. Oecologia 65: 487-491.