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Inbreeding and outbreeding depression in *Daphnia*

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Abstract. Egg-to-adult viability of sexual offspring in *Daphnia magna* is lower for selfed (average: 43.0%) than for outcrossed families (average: 74.7%). This suggests that intraclonal mating is not the rule in *Daphnia* populations. For a given family, hatching rate of eggs resulting from interpopulation crosses is lower than for intrapopulation crosses. This breakdown in hatching responses may result in the effective gene flow between *Daphnia* populations being severely reduced, offering an explanation for the apparent paradox of genetic differentiation of *Daphnia* populations in spite of efficient dispersal.

Key words: *Daphnia* – Inbreeding depression – Outbreeding depression – Hatching – Local differentiation

In the past 20 years, numerous studies have analysed the genetic structure of natural *Daphnia* populations by allozyme electrophoresis (e.g. Hebert 1974, 1987; Lynch 1983; Jacobs 1990). Often striking differences have been observed in the genetic composition of different populations, even when they are separated by only a short distance (a few meters in Hebert 1974). Extensive local differentiation has also been observed in a variety of other pond-dwelling invertebrates (Boileau et al. 1992) as well as in marine intertidal copepods (Burton 1987; Brown 1991).

The analysis of genetic variation in ecologically relevant characters has added to the evidence for local differentiation in zooplankton populations. Variability in timing of diapause in the pond-dwelling calanoid copepod *Diaptomus sanguinensis* has been shown to have a genetic component, and is locally adjusted to pond characteristics (Hairston and Olds 1984). Weider and Hebert (1987) reported between-population differences in salinity tolerance in obligately parthenogenetic *D. pulex* on a microgeographical scale. The results of Parejko and Dodson (1991) and De Meester (1993) indicate that *Daphnia* clones isolated from habitats without predators (*Chaoborus* and fish, respectively) tend to be less responsive to the presence of chemicals produced by these predators than clones from habitats in which predators are present. Though local differentiation has been mainly attributed to chance effects (founder effects; Boileau et al. 1992), and fluctuating selection (Lynch 1987), the abovementioned studies indicate that genetic variation in specific traits tends to be correlated with habitat characteristics. This points to differential selection and local adaptation as an important force for genetic differentiation between zooplankton populations.

Dispersal in *Daphnia* is passive: resting eggs are deposited in a protective envelope (ephippium), and can be transported from habitat to habitat by biological agents such as waterfowl (Proctor 1964; Mellors 1975). Several studies have reported rapid colonization of new ponds by *Daphnia* (Hebert and Moran 1980; Fryer 1985; Pajunen 1986). These observations point to a potentially high level of gene flow between *Daphnia* populations, contrasting with the observations on genetic differentiation. This paradox can be solved by hypothesizing that the chance of a given genotype successfully colonizing a habitat is severely reduced when the habitat is already occupied by conspecifics. Hebert and Moran (1980) stated that this reduction of effective gene flow might be mediated through competition with resident genotypes. The results of an *in situ* competition experiment by Korpelainen (1986) provides some empirical support for this hypothesis. The results of her study indicate competitive superiority of the resident genotypes, and therefore add support for local adaptation.

In the present study, I compare hatching rate and viability of sexual offspring between selfed and outcrossed *D. magna* families, differentiating between intrapopulation and interpopulation crosses. In addition, hatching rates and viability of hatchlings was determined for ephippia collected from two *D. magna* populations. The data corroborate earlier reports on inbreeding depression in *Daphnia* (Innes 1989), but also show that the success rate of a second colonizer may be reduced by a lowered hatching rate of its sexual offspring.

Material and methods

Origin of the clones

Clones were derived from four different habitats, their habitat being indicated by the first character of their name: B-clones were isolated from Lake Blankaart, a shallow, eutrophic lake (about 30 ha; Western Flanders), C-clones were derived from a city pond in Citadelpark (Gent, Eastern Flanders), P-clones from Driehoekvijver, a pond near the river Schelde (Heusden, Eastern Flanders), and R-clones were isolated from the Ringvaart, a highly eutrophic canal (Gent). Lake Blankaart (Western Flanders) is situated at about 80 km from Gent, the distance between the other habitats is less than 7 km.

The clones isolated from natural populations were used in various intra- and interclonal crosses. The methods used to induce sexual mixis and obtain sexual eggs of known origin are described in De Meester (1991). As males in cladocerans are genetically identical to females (Zaffagnini 1987), intraclonal mixis is genetically equivalent to selfing ("clonal selfing"). In total, 22 intraclonal and 12 interclonal families were obtained. Offspring clones were given a compound name (see De Meester 1991), consisting of the name of the mother clone, followed by the name of the father clone, and a number, indicating the chronological order of hatching. In the case of intraclonal sexual offspring, the name of the parent clone is followed by a number. In the following, families will be referred to by the name of the parent clone(s).

Hatching, mortality estimates

The hatching procedure used in the present study is the same as described in De Meester and De Jager (in press a). It is a modification of the manual decapsulation technique used by Schwartz and Hebert (1987). Ephippia were collected within 48 h after being deposited, and were stored wet in the dark, at 4° C (range 1° C) for at least 2 months. Hatching treatment consisted of manual decapsulation, refreshment of the medium (dechlorinated tap-water), and incubation in a well illuminated (103×1^{-3} W m^{-2}; 14 h light/10 h dark photoperiod) and temperature-buffered (20° C, range 1° C) room. The incubated eggs were checked daily for 18 days, and the number of hatchlings, dead (deformed) embryos and degenerated eggs was recorded. Fresh medium was added at day 6, and thereafter every other day.

Hatchlings were inoculated in 250-ml culture jars. Medium consisted of 4/5 fresh dechlorinated tap-water and 1/5 of 'conditioned' medium (from a culture with a vigorous *Daphnia* population at low density, filtered over 40 µm). Using partially conditioned medium enhanced survival (De Meester, pers. obs.). Every 2-nd day, 1/5 of the medium was refreshened, and food was supplied. Food consisted of unicellular algae *(Scenedesmus acutus)* grown in batch cultures. In addition, one drop of horse manure extract was added as a supply of bacteria. Food concentration was kept relatively low (5×10^4–1×10^5 *Scenedesmus* cells ml^{-1}) for the first 4 days, and thereafter was restored daily to 1.5–2.5×10^5 *Scenedesmus* cells ml^{-1}).

Hatching rate (%H) is defined as the percentage of eggs that responded to the hatching stimuli, including dead embryos, but not degenerated eggs. Mortality of the eggs (MEGG) is defined as the percentage of eggs that degenerated after applying the hatching stimuli. Mortality of embryos (MEM) is determined as the percentage of developing embryos that did not develop into living hatchlings. Whereas there is no certainty that degenerated eggs (MEGG) were responding to the hatching stimuli, MEM refers to the proportion of developing embryos that failed to complete development. Mortality of juveniles (MJUV) was detemined as the proportion of hatchlings that did not survive until maturity (primiparae). Total mortality was also calculated, excluding (M1) and including (M2) degenerated eggs. For some families for which a relatively large number of eggs were incubated, observations on mortality were obtained for replicate groups of 20–30 responsive eggs (i.e. eggs that degenerated or developed). One-way ANOVAs were carried out on the data of the selfed families (C_1, P_1, $P_1 32$, $P_1 81$ and R_2) for which several independent observations were available, using the SYSTAT program (Wilkinson 1990).

Ephippia from natural populations

I collected *D. magna* ephippia along the shoreline of Lake Blankaart (24 September 1989) and the Driehoekvijver (20 June 1991). They were stored wet in the dark at 4° C, and were decapsulated on 10 July 1992. Altogether 120 eggs from the Blankaart population and 60 eggs from the Driehoekvijver population were treated. The hatching procedure was essentially as described above. However, only half of the eggs (60 from the Blankaart, 30 from the Driehoekvijver) were incubated in dechlorinated tap-water, whereas the other eggs were incubated in "oxygen-enriched" water, prepared by dissolving a JBL oxygen tablet in 150 ml dechlorinated tap-water. Hatching rate and mortality of the eggs, embryos and hatchlings was determined as described above.

Table 1. Mortality of the offspring of intra- and interclonal *Daphnia magna* families

Family	N	MEGG	MEM	MJUV	M1	M2
INTRA						
B_5	27	18.5	0.0	18.2	18.2	33.3
C_1	221	12.2	13.9	44.3	52.1	57.9
P_1	169	13.0	4.1	56.0	57.8	63.3
$P_1(F_2)$	348	15.5	19.4	62.9	70.1	74.7
$P_1(F_3)$	13	15.4	18.2	55.6	63.6	69.2
R_2	79	0.0	1.3	14.1	15.2	15.2
$R_2(F_2)$	60	31.2	9.5	52.6	57.2	70.5
Total[a]	996	13.6	12.7	50.1	56.5	62.4
						(56.96 ± 16.23)[b]
INTER						
$(C_1 \cdot C_2)$[c]	42	11.6	2.6	8.1	10.5	20.9
$(C_1 \cdot P_1)$	27	3.7	19.2	23.8	38.5	40.7
$(C_1 \cdot R_1)$	18	0.0	5.6	17.7	22.2	22.2
$P_1 \cdot P_2$	82	30.5	0.0	15.8	15.8	41.5
$(R_1 \cdot P_1)$	26	3.9	0.0	28.0	28.0	30.8
$R_2 \cdot R_1$	17	0.0	0.0	11.8	11.8	11.8
$R_1P_17 \cdot C_1$	17	9.1	10.0	5.6	15.0	22.7
$R_1P_17 \cdot P_1$	17	5.9	0.0	6.2	6.3	11.8
Total[a]	289	13.5	4.4	14.9	18.3	29.3
						(25.30 ± 8.16)[b]

N: number of eggs that initiated development or degenerated after application of the hatching stimuli; MEGG: % mortality of eggs [% eggs degenerated/(eggs degenerating or developing into embryos)]; MEM: % mortality of embryos; MJUV: % mortality among hatchlings; M1: total mortality (%), excluding degenerated eggs; M2: total mortality (%), including degenerated eggs. F_2 and F_3 refer to successive generations of selfing [averages of several families: 4 in $P_1(F_2)$, 5 in $P_1(F_3)$, 6 in $R_2(F_2)$]

[a] Totals refer to all intra- and interclonal families which were analysed in the hatching experiments (including a few families with a low number of eggs, not mentioned separately in the table)

[b] Averages ± 2 SE for totals (M2) are based on all families with > 20 hatchlings

[c] Interclonal families in parentheses refer to the pooled data of reciprocal crosses

Results

Mortality of offspring clones

The data in Table 1 suggest that mortality after selfing is family-dependent, with some families showing a far lower mortality than others. One-way ANOVAs with the data of the selfed families (C_1, P_1, P_132, P_181 and R_2) for which several independent observations were available (untransformed data; variances were homogenous by Bartlett's test, $P > 0.3$ in all cases) revealed a significant effect of family on total mortality (M1 and M2; $P < 0.001$) as well as on the mortality of embryos (MEM; $P < 0.05$) and juveniles (MJUV; $P < 0.05$), but not on the proportion of degenerated eggs (MEGG). Tukey pairwise comparisons indicated that this effect was in all cases due to a consistently lower mortality of the offspring resulting from selfing clone R_2.

Mortality of sexual offspring clones from clonal selfing (57.0 ± 16.2) is on average more than twice as high than mortality of offspring clones from interclonal crosses (25.3 ± 8.2; Table 1). This also holds for a comparison of specific families: the mortality observed for the interclonal crosses of families C_1 and P_1 ($M2 = 40.7\%$) is 67% of that of the offspring resulting from selfing the same clones (average $M2 = 60.6\%$). Mortality (M2) of selfed offspring from clone R_1 is 69.6% (based on 23 eggs, not mentioned in Table 1 because an extra hatching stimulus was needed to hatch these eggs), and it is again observed that all interclonal crosses with R_1 as one of the parents have a lower mortality than expected from the mortality of the families resulting from selfing the parents.

The percentage of eggs that degenerated after applying the hatching stimuli is similar for both selfed and intercrossed families, but the percentage of embryos that complete development, and the percentage of hatchlings surviving to maturity is much higher in interclonal families than in selfed ones. Therefore, differences in M1 are larger than differences in M2.

Hatching efficiency

The overall hatching rate of interclonal sexual eggs (19.78%, on a total of 278 eggs) is lower than that of eggs resulting from selfing (38.57%, 1260 eggs). As hatching rate is family-dependent (De Meester and De Jager in press a), Table 2 reports the results of specific families. Hatching rate of eggs resulting from crossing two clones derived from different natural populations is lower than that of eggs resulting from crossing two clones from the same population in 10 out of the 11 interpopulation families studied ($P < 0.01$ by the Wilcoxon matched-pairs signed-ranks test). Hatching rates of eggs resulting from interpopulation crosses tended also to be lower than of eggs resulting from selfing (lower hatching rates in 8 out of 11 families, but the difference was not statistically significant by the Wilcoxon matched-pairs signed-ranks test). Finally, hatching rate of eggs of intrapopulation crosses is higher than that of selfed families in all four family-pairs) (Table 2).

Table 2. Hatching rate (%) of the sexual eggs of intra- and interclonal *Daphnia magna* families

	Self (F_1)	Self (F_2)	Within	Among
C_1	19.8		36.5 (1)	P : 11.3 (2); R: 8.3 (2)
P_1	69.8	53.6 (4)	57.6 (1)	C: 18.2 (3); R: 34.5 (1)
R_1	0.0[a]	4.0 (1)	42.5 (1)	C: 8.3 (2); P : 34.5 (1)
R_2	23.2	38.6 (4)	42.5 (1)	

Self (F_1): sexual eggs resulting from selfing the parent clone; Self (F_2): sexual eggs from second generation of selfing; Within: interclonal crosses with parent clones derived from the same population; Among: interclonal crosses with parent clones derived from different population (which population is identified by character). Numbers in parentheses refer to the number of families involved (number of eggs per family is >25)

[a] 0.0% hatching rate refers to the hatching stimuli as applied in the present study; application of other hatching stimuli has yielded some offspring clones for this family

Ephippia from natural populations

Hatching rate and mortality of the sexual offspring is very similar for the ephippia isolated from two different populations (Table 3). Incubation in oxygen-enriched water results in a higher hatching rate, but also in a higher mortality of eggs and embryos. With ephippia

Table 3. Hatching rate and mortality of the offspring from *D. magna* sexual eggs isolated from natural populations (Blankaart and Driehoekvijver)

Population	*n*	N	N°	%H	MEGG	MEM	MJUV	M1	M2
Blankaart									
A	60	37	36	60.0	2.7	0.0	14.8	19.8	24.2
B	60	54	45	90.0	9.3	8.2			
Driehoekvijver									
A	30	20	20	66.7	0.0	0.0	17.1	24.4	27.7
B	30	27	21	90.0	7.4	16.0			

A: eggs incubated in dechlorinated tap-water; B: eggs incubated in dechlorinated tap-water in which a JBL oxygen tablet was dissolved (one tablet in 150 ml); n: number of eggs incubated; N: number of eggs that responded to application of the hatching stimuli; N°: number of living hatchlings; %H: hatching rate; MEGG: % mortality of eggs; MEM: % mortality of embryos; MJUV: % mortality among hatchlings; M1: total mortality (%), excluding degenerated eggs; M2: total mortality (%), including degenerated eggs from the Driehoekvijver, the two methods yield the same number of living hatchlings. Mortality of the sexual offspring of ephippia collected from both natural populations is similar, and relatively low: 24.2% for the Blankaart, and 27.7% for the Driehoekvijver. This value is well within the range of mortalities observed for laboratory crosses between different clones (11.8–41.4%), and far lower than the average mortality observed in the laboratory for selfed families (57.0%).

Discussion

The inbreeding depression observed in *Daphnia magna* is in concordance with the results presented by Innes (1989) on *D. obtusa*. His data on survivorship include mortality of embryos and hatchlings, and are therefore comparable to the present M1 mortality scores. Innes (1989) reports an average mortality of 14% in interclonal and 57% in intraclonal families, which is very similar to my observations (18 and 56%, respectively). Survival of parthenogenetic offspring of the parent clones is invariably high (>90%; De Meester pers. obs.). The difference in viability between intra- and interclonal sexual offspring can most easily be explained by reduced viability after selfing due to the expression of deleterious mutations, though reduced fitness due to homozygosity *per se* can not be ruled out. Inbreeding depression upon selfing was also observed by Banta and Wood (1928) in *D. longispina* and by Birky (1967) in the rotifer *Asplanchna brightwelli*.

Whereas the difference in mortality of embryos (MEM) and ex-ephippial juveniles (MJUV) between intra- and interclonal crosses is well-expressed, the two types of families do not differ in the proportion of eggs degenerating after application of the hatching stimuli. However, as mentioned, there is no certainty whether degeneration of eggs after application of hatching stimuli is related to inferior viability of the eggs upon initiating development.

The family-dependent survivorship of offspring resulting from clonal selfing, also observed in the present study, was interpreted by Innes (1989) as indicating differences in the age of the parent clones. However, in cyclic parthenogenetic *Daphnia* (such as *D. obtusa* and *D. magna*), and in contrast to obligately parthenogenetic daphniids, a much more likely explanation is that the observed inbreeding depression indicates that selfing (intraclonal mating) is not a common mating strategy in natural *Daphnia* populations. My results therefore suggest that *Daphnia* populations are not severely inbred, though they do not imply that mating is completely random. In spite of general statements inferred from electrophoretic data (Lynch 1983; Hebert 1987), the extent to which mating in *Daphnia* populations is random certainly needs further investigation. Observations on sex-related differences in phototactic behaviour in *Daphnia magna* suggest a mechanism promoting random mating between spatially segregated clones, but at the same time indicate that assortative mating may be predominant in negatively phototactic clones, which tend to be highly specialized (De Meester 1992).

Though they do not provide conclusive evidence, my observations on mortality of sexual offspring from ephippia collected from natural habitats also indicate the prevalence of interclonal crosses rather than selfing in natural populations. The mortality of hatchlings observed in the present study (14.8% for the Blankaart sample, 17.1% for the Driehoekvijver) is lower than that reported by Carvalho and Wolf (1989) for lake *Daphnia* (*D. galeata, D. hyalina, D. cucullata,* and *D. galeata* × *D. hyalina* hybrids; average mortality of ex-ephippial juveniles: 35%). However, this higher mortality most probably is the result of the lake *Daphnia* being more difficult to culture and more vulnerable to manipulation than *D. magna*, and should not be used to infer a higher incidence of selfing in these species.

Though egg-to-adult survivorship is the character most often used in studies on inbreeding depression (Charlesworth and Charlesworth 1987), it is a partial and therefore crude measure of fitness. Life table experiments would probably reveal more subtle reductions in fitness of offspring from intraclonal matings compared to interclonal offspring. For instance, Innes (1989) observed that the growth of ex-ephippial neonates hatching from inbred eggs is slower than that of hatchlings from crossed eggs. Similarly, breakdown of the hatching response is probably only one of the aspects of outbreeding depression related to interpopulation crosses. Outbreeding depression might be expected in all characters for which particular populations show a tendency for local adaptation, and the more so if intermediate values (or intermediate reaction norms if responses to environmental cues are involved) have no adaptive value. Such subtle effects will, however, often be very difficult to measure in the laboratory, as they may be strongly dependent on the environmental conditions provided.

The differences in hatching rates between families is probably due to a differential response to the hatching cue (Schwartz and Hebert 1987) rather than to a differential capability of the eggs to hatch (viability). Indeed, the eggs retain their normal shape, and some may hatch after a second stimulus (De Meester and De Jager in press b).

The reduced hatching rate of sexual eggs resulting from interpopulation crosses may strongly reduce the chances of a genotype establishing itself in a habitat as a second colonist, even if the immigrant genotype is successful in competing with resident genotypes during the parthenogenetic phase. Unless the propagules of immigrant genotypes include several successful genotypes, mating partners of the immigrant genotype will almost invariably be resident genotypes. Outbreeding depression, as evidenced by the reduced hatching rate of interpopulation crosses, may then be an important factor reducing the effective gene flow between (neighbouring) *Daphnia* populations.

In the case of initially successful immigrant genotypes, a substantial proportion of mating partners may be clone-mates. My observations on inbreeding depression suggest that in this case too, however, production of sexual offspring will be a bottleneck for the immigrant genotype compared to the resident clones. In addition to the observation that immigrant genotypes may be competitively inferior (Korpelainen 1986), my results indicate that effective gene flow between *Daphnia* populations may also be reduced by inferior fitness of the sexual offspring produced by the immigrant clones. My observations may therefore be important in explaining the extensive genetic differentiation of *Daphnia* populations in spite of high dispersal capacities, illustrated by the rapid colonization of new ponds. Though genetic differences between *Daphnia* populations may for a substantial part be due to chance effects (Boileau et al. 1992) and fluctuating selection (Lynch 1987), these mechanisms can not explain local adaptation as observed for quantitative characters (e.g. Parejko and Dodson 1991).

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