

Aspects of mating, reproduction, and co-occurrence in three freshwater calanoid copepods

PATRICIA CHOW-FRASER* and EDWARD J. MALY Concordia University,
Department of Biology, Montreal, Quebec, Canada

SUMMARY. 1. We examined the seasonal distributions and mating behaviour of *Epischura lacustris*, *Diaptomus minutus* and *Diaptomus oregonensis* to evaluate several proposed mechanisms for reproductive isolation. There was no divergence in onset of maturity or timing of breeding periods between sympatric diaptomid populations; size displacement between co-occurring diaptomid species did not result in concomitant reduction in mating frequency between heterospecific pairs in the laboratory.

2. Divergence in mating behaviour was supported by observations that, in conspecific mating experiments, males of both diaptomid species discriminated between females bearing ripened ovaries (gravid) and those that did not (non-gravid), and that, in heterospecific trials, males either mated indiscriminantly with all females, or engaged in mating very infrequently.

3. In mating experiments with *Epischura*, males did not discriminate between gravid and non-gravid females, but mated exclusively with virgins of a specific relative size. Divergence in timing of breeding seasons between *Epischura* and the two diaptomid species suggests that even if mating between genera is possible, it does not occur often in nature.

4. We discuss the adaptive significance of different reproductive strategies of these three copepods and speculate on mechanisms that allow for coexistence of the closely related taxa in nature.

Introduction

Reproductive isolation is prerequisite to maintenance of taxonomically distinct populations when closely related species co-occur in nature. Not surprisingly, a great deal of research has been conducted to uncover mechanisms that

*Present address: University of Alberta, Department of Zoology, Edmonton, Alberta, Canada T6G 2E9.

Correspondence: Dr E. J. Maly, Department of Biology, Concordia University, 1455 de Maisonneuve Blvd. W., Montreal, Quebec, Canada H3G 1M8.

lead to reproductive isolation. But while mechanisms are often proposed, the task of validating such hypotheses is slow to follow suit. In this study, we try to remedy this situation by using information on the reproductive biology of three calanoid copepods to evaluate applicability of several proposed mechanisms.

The copepods studied include *Epischura lacustris* Forbes, *Diaptomus minutus* Lilljeborg, and *D. oregonensis* Lilljeborg. These copepods frequently assort in pairs in lakes of south-eastern Quebec (Maly, 1984), and are three of the

most commonly occurring crustaceans in glaciated eastern North America (Carter *et al.*, 1980). They share a similar ecology in lakes (Sandercock, 1967; Rigler & Langford, 1967, being mainly herbivorous in many of their life-stages (Chow-Fraser, 1986; Chow-Fraser & Wong, 1986). The two diaptomids are similar in size, and when they are found sympatrically, sometimes exhibit size displacement (Maly, 1984) or partitioning in their temporal or spatial distributions (Sandercock, 1967; Rigler & Langford, 1967).

Divergence in size and/or distribution has sometimes been viewed as evidence that inter-specific competition has been a selective pressure (Hutchinson, 1951; Cole, 1961; Maly, 1984). In this study we consider an alternative view, that size displacement has evolved to prevent interbreeding between very similar-sized species. As such, it is a form of morphological divergence that has already been proposed as a mechanism for reproductive isolation among calanoid copepods (Davis, 1961; Fleminger, 1967, 1975; Blades & Youngbluth, 1980; Jacoby & Youngbluth, 1983).

Another mechanism that may apply to calanoid copepods is divergence in behaviour, in addition to, or instead of morphology. This is based on the observations that: (1) many calanoid copepods display species-specific mating rituals (Blades & Youngbluth, 1980; Jacoby & Youngbluth, 1983), and (2) that several diaptomid species selectively engage in pre-copulatory coupling with gravid versus non-gravid females (Watras, 1983). Since in *Diaptomus* only gravid females produce viable clutches when mated (Watras, 1983), these observations suggest that males have not only evolved to recognize females of their own species, but also to discriminate reproductively receptive individuals from those that are not. Such discriminatory power may effectively serve to prevent hybridization between species, as long as males do not mate indiscriminantly with gravid females of any species.

A third mechanism that may apply is divergence in timing of reproductive events. This hypothesis maintains that species that do not exhibit divergence in behaviour or morphology, may still experience reproductive isolation if there is temporal partitioning in their breeding periods (Davis, 1961; Sandercock, 1967; Maly, 1976; Jacoby & Youngbluth, 1983).

To evaluate these proposed mechanisms, we conducted laboratory mating experiments with lake-resident populations of the three copepod taxa. The factors we considered include relative size of mating pairs, reproductive condition of females (gravid versus non-gravid), and taxonomic affiliation of each mating partner. In addition, we compared seasonal distributions of allopatric and sympatric populations and relate these findings to differences in reproductive and life history characteristics.

Methods

We selected four lakes, Trouser, Nick, Sally and Moulin Lakes, in which the three copepod taxa were found in various combinations. Nick and Trouser Lakes contained all three, Sally contained *Epischura* and *D. minutus*, and Moulin contained only *D. oregonensis*. These lakes are located in the vicinity of Montreal and their physico-chemical characteristics have been published elsewhere (Maly, 1984).

Field collection

Quantitative zooplankton samples (2-litre Schindler-Patalas traps; mesh size 110 μm) were collected from top and bottom strata at several locations in each lake. Ten replicate samples were taken from each lake and were preserved in the field with 4% formalin solution. In total, six sampling trips were made from the latter part of May to the end of August in 1986. In the laboratory, adult stages of each copepod taxon were identified and enumerated with the aid of a dissecting microscope.

Qualitative samples were also collected in diagonal net tows (mesh size 80 μm) and preserved with formalin solution in the field. In the laboratory, an aliquot of each sample was withdrawn and examined under a dissecting microscope. Adult and copepodid stages of each diaptomid species were identified and enumerated. Whenever possible, at least 100 individuals of each taxon were counted in each sample. From this, percentage composition data were calculated for copepod life-stages.

Mating experiments

Copepods were collected in diagonal net tows from each of the four lakes at various times

during the ice-free season. They were kept in lakewater in 30-litre plastic carboys and transported back to the laboratory, usually within 4–6 h of collection. Animals from each lake were transferred to separate aerated glass aquaria, kept at between 20 and 22°C, and subjected to a natural photoperiod. Copepods were fed aliquots of an algal suspension of *Chlorella* sp., *Chlamydomonas* sp., *Ankistrodesmus* sp., *Scenedesmus* sp., and unidentified microflagellates that had been cultured in a goldfish aquarium. The algal mixture was added immediately after animals were placed in aquaria, and daily thereafter until experiments were terminated. We only used animals that were actively swimming and that had been kept in captivity for less than 3 days. Animals from the maintenance aquaria were isolated in depression plates with the aid of a dissecting microscope. One male and one non-egg-bearing female with no attached spermatophores were then placed into a 1000 ml holding beaker and then into a clear plastic vial containing approximately 30 ml of 64- μ m-filtered lakewater. The source of lakewater was the lake from which animals were obtained; when animals from two different lakes were involved, lakewater from one lake was arbitrarily employed. The reproductive condition of the female was noted as gravid or non-gravid; a female was considered gravid when most of the oocytes had acquired a dark coloration. Vials were kept under the same conditions as maintenance aquaria (but not aerated). After a period of from 3 to 24 h, copepods were killed and preserved with the addition of 10 drops of 37% formalin solution. Whenever possible, experiments with five to ten replicate pairs were performed simultaneously. Depending on availability of animals in the maintenance aquaria, isolation and sorting consumed from 1 to 4 h for every ten replicates run. Pairs of animals from

the same and different lakes were included in these experiments. In total, 744 experiments were conducted (see Table 1).

Presence of a spermatophore or spermatophores on female genital pore or urosome, male antennule, or bottom of the vial was considered evidence for a mating attempt. This method of scoring does not take into consideration attempts that might have been made but that did not result in extrusion of spermatophores, nor does it distinguish between single and multiple transfers. No effort was made to determine whether or not the spermatophore was full or empty. We noted whether or not females were bearing eggs at the end of the experiment, and measured the prosome length of copepods to calculate a size-ratio (female/male prosome length).

In a series of experiments we determined the effect of experimental duration on mating frequency. Results from 3-h and 24-h experiments were statistically compared with Chi-square analysis. We found no evidence that length of experiment had any effect on the data ($\chi^2=0.106$; $P>0.75$). Secondly, by monitoring pairs of copepods in 1 ml micropipette plates, we confirmed that mating, if attempted at all, took place within the first 2 h of coupling. Thus, duration of the mating experiment was not an important consideration here, since duration of all experiments exceeded 2 h.

Although *Epischura* were present in Nick and Trouser Lake, they were so rarely found that mating experiments were only performed with animals from Sally Lake. For the same reason, very few experiments were conducted with *D. minutus* from Nick Lake. There was also a disproportionate representation of both allopatric diaptomid populations in the dataset; sympatric populations were generally found in low numbers except in July.

TABLE 1. Summary scores of mating experiments. DM=*D. minutus*; DO=*D. oregonensis*; NS=not significant ($P>0.05$).

Male species	Female species	Gravid		Not gravid		Chi-square	P
		Mated	Not mated	Mated	Not mated		
DM	DM	18	20	14	125	27.57	0.001
DO	DO	25	27	25	123	19.96	0.001
DO	DM	7	32	30	173	0.14	NS
DM	DO	1	23	4	97	0.00	NS

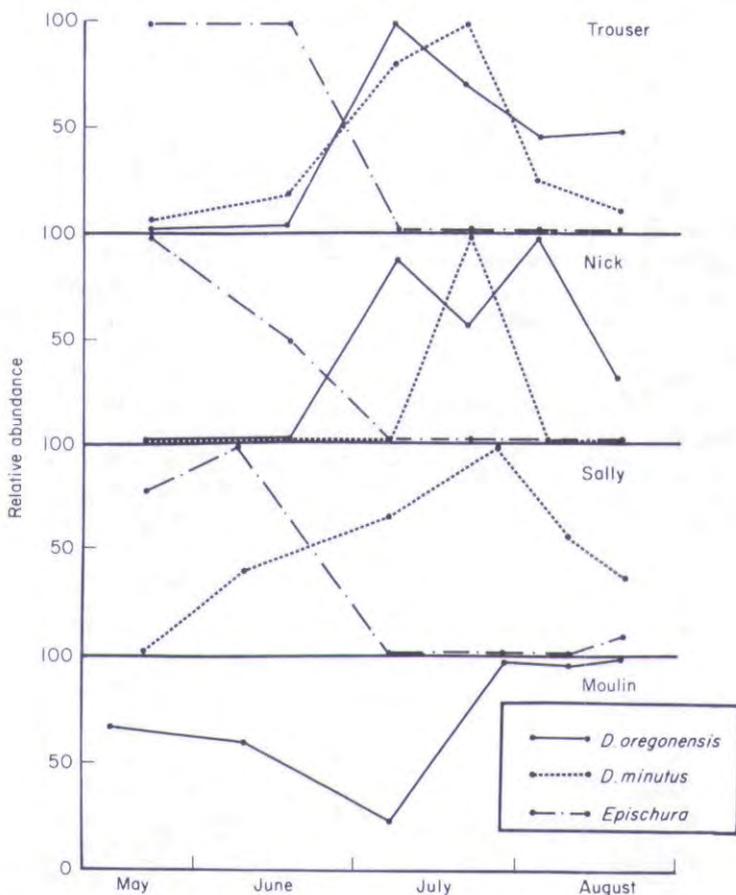
We analysed mating frequency data by means of appropriate Chi-square tests (Sokal & Rohlf, 1981). To test for mutual independence of size ratio, reproductive condition and frequency of mating attempts; we used a three-dimensional contingency table (Zar, 1984).

Life history and reproductive characteristics

Copepods were captured and maintained as in mating experiments. As soon as it was convenient (usually within 6 h of collection), egg-bearing females were isolated and placed individually in plastic vials containing 30 ml of filtered lakewater. Vials were also kept under the same conditions as in maintenance aquaria. We recorded whether or not females were gravid, and then determined their clutch size. Condition of the females was monitored at approximately the

same time each day until they expired. We recorded whether she still carried eggs and/or was gravid, and whether or not nauplii were visible if she no longer carried eggs. Nauplii, if present, were subsequently removed. Water in the vials was changed approximately every 5 days, and approximately 1 ml of algal mixture (as above) was added two or three times per week. We measured prosome length of the female upon her death and discarded contents of the vial. These experiments were replicated twice (once each in July and August) for Nick and Trouser animals and three times (one each in June, July and August) for Moulin and Sally Lakes.

To determine the period between final moult and initial gravid condition, we isolated thirty to fifty late copepodid stages (C3 or C4) and placed them individually into experimental vials as above. They were also held under the same



FIGS. 1. Relative abundance of adult calanoid copepods plotted against sampling date. Values are percentage of the maximum observed for each species.

conditions as for egg-bearing females. Animals were monitored daily for production of moults. Upon their final moult, females were scored daily as gravid or non-gravid. For males, only time of death was noted. Owing to the lengthy duration of these experiments, they were not repeated.

Results

Seasonal distribution

In Nick and Trouser Lakes, where the diaptomid species occur sympatrically, seasonal maxima for both species of adults occurred during July (Fig. 1). In Sally and Moulin Lakes, where they occur allopatrically, seasonal maxima

were similarly observed in July. Thus, females of both diaptomid species matured in large numbers in July, regardless of presence of congeneric species.

By contrast, adult *Epischura* exhibited their seasonal maxima in May and June, and quickly disappeared in July; by mid-August a second generation of adults appeared but was usually found in low numbers. This pattern of abundance was noted regardless of co-occurrence of *Diaptomus*.

Examination of percentage composition for the various diaptomid populations partially confirmed the trend noted above (Fig. 2). There was no divergence in onset of maturity between sympatric diaptomid populations. Peaks in the relative abundance of adult diaptomid species in

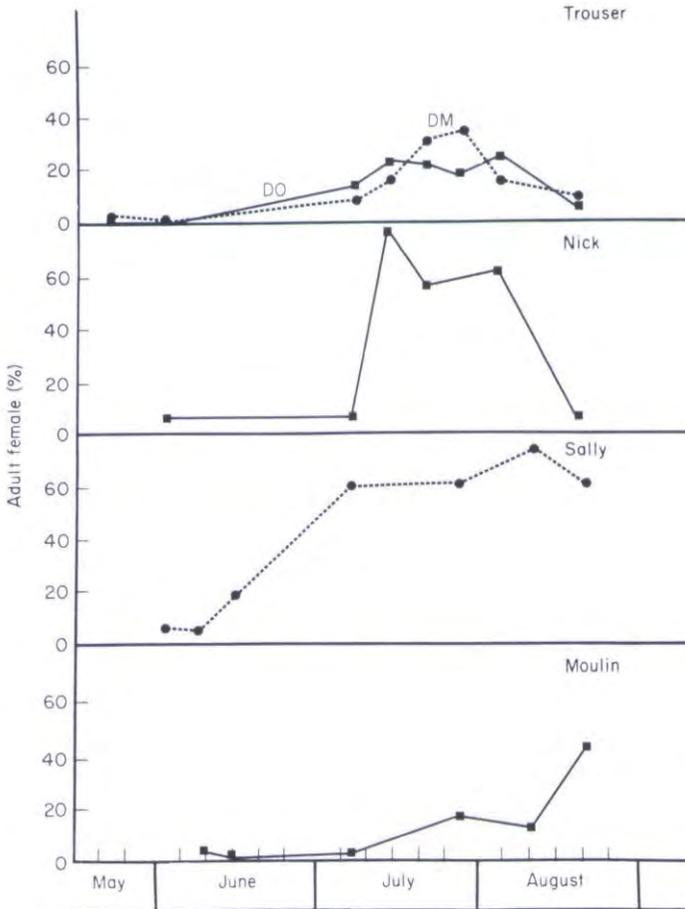


FIG. 2. Percentage of the total copepodid and adult population that was adult female in samples plotted against sampling date. *D. minutus* in Nick Lake were present in negligible numbers throughout the summer.

Trouser Lake overlapped in mid-July; there were unfortunately insufficient data to substantiate this trend in Nick Lake. By comparison, there were no peaks in percentage composition data corresponding to the allopatric populations. In both Sally and Moulin Lake, percentage composition of adults in samples did not decline during the summer after the initial increase in mid-June.

Conspecific mating between diptomids

Experiments were conducted with animals collected from all four lakes over a 3-month period. Before we pooled data by species (to increase sample size for statistical rigour), we first investigated whether or not frequency of mating attempted was affected by time of year. With some variation, approximately the same proportion of animals attempted to mate in each month, proportions usually ranging from 28% to 33% for *D. oregonensis* (Fig. 3a) and from 23% to 26% for *D. minutus* (Fig. 3b). Effect of lake

origin was also minor, since mating frequency corresponding to different lakes were very similar.

When low mating frequencies were recorded, corresponding proportion of gravid animals in samples were also low (Fig. 3c and d), suggesting that effect of reproductive condition superceded any effect of season or lake origin on frequency of mating attempts. Since this analysis demonstrated that time of year had minimal effect on mating data, we proceeded to pool data by species, and test the effect of reproductive condition and size-ratio on frequency of mating attempts.

The three-dimensional contingency table, appropriate for testing mutual independence of three variables (Zar, 1984) was used to examine the simultaneous effects of sex-size-ratio and reproductive condition on mating data. The data were pooled by species, regardless of lake origin. In both cases the null hypothesis of mutual independence was rejected ($\chi^2=70.32$, $df=34$, $P<0.001$ for *D. minutus*; $\chi^2=57.85$,

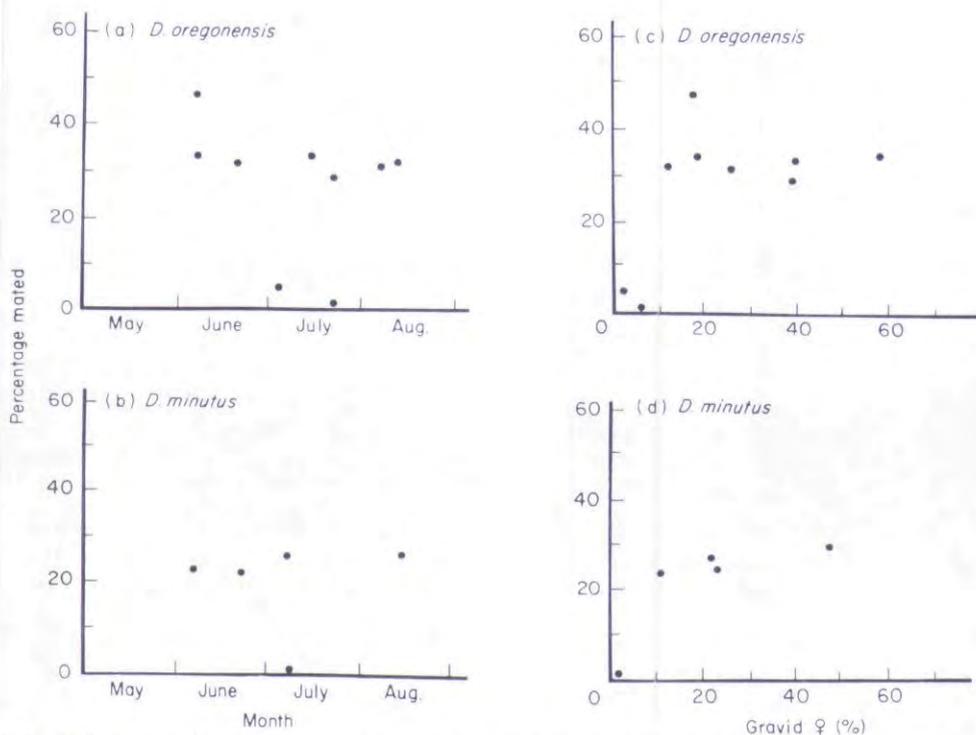


FIG. 3. (a) Percentage of males that attempted to mate plotted against experiment date for *Diaptomus oregonensis* data. (b) Same as (a) but for *D. minutus* data. (c) Percentage of males that attempted to mate plotted as a function of per cent gravid females in sample for *D. oregonensis* data. (d) Same as (c) but for *D. minutus* data.

df=40, $P < 0.05$ for *D. oregonensis*). We then proceeded to determine the effects of each variable separately.

We tested to see if reproductive condition alone could influence mating frequency. For both species of *Diatomus*, without taking size-ratio into consideration, a statistically higher proportion of males attempted to mate with gravid than with non-gravid females (Table 1), thus confirming the strong effect of reproductive condition on mating attempts noted earlier (Fig. 3c and d).

Next, we tested if size-ratio alone could influ-

ence mating frequency. When data were pooled by species, regardless of lake origin and reproductive condition, we found no significant effect of sex-size-ratio on frequency of mating attempts for either species; however, once we accounted for reproductive condition, we found a significant effect for gravid individuals of *D. minutus* (Table 2, Fig. 4). By contrast, size-ratio did not significantly affect mating frequency of *D. oregonensis* males although somewhat fewer attempts were recorded at size ratios near unity, at least over the range of ratios examined (Fig. 4).

TABLE 2. Summary statistics from tests of independence of mating frequency on size-ratio. DM=*D. minutus*; DO=*D. oregonensis*; G=gravid; NG=non-gravid; NS=non-significant ($P > 0.05$).

Species	Reproductive condition	n	DF	Chi-square	P
DM	G+NG	169	5	8.74	NS
	G	38	5	12.08	0.05
	NG	131	5	4.41	NS
DO	G+NG	200	6	5.56	NS
	G	52	6	1.28	NS
	NG	148	6	10.18	NS

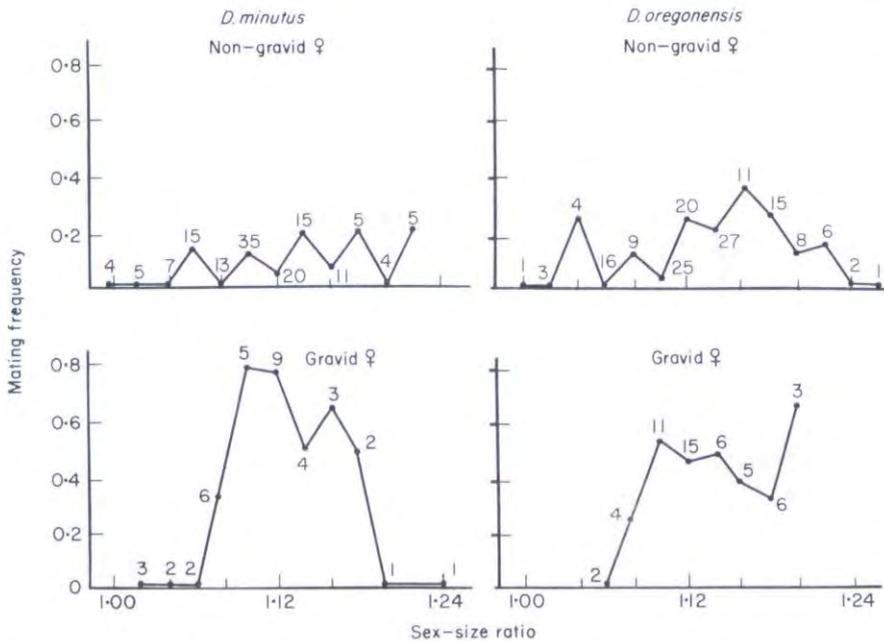


FIG. 4. Frequency of mating attempts plotted as a function of corresponding size-ratio. Numbers in the body of the figure indicate total number of experiments used to calculate frequencies.

Heterospecific mating between diaptomids

We first tested results of heterospecific mating experiments for mutual independence of reproductive condition and frequency of mating attempts (Table 1). The proportion of males that attempted to mate with gravid females did not differ significantly from that with non-gravid females, indicating that gravid condition was not an important cue for heterospecific coupling. In general, frequency of mating attempts was low compared with those of conspecific mating experiments (Table 1), especially when *D. minutus* males were paired with *D. oregonensis* females.

We grouped the pooled data for heterospecific mating between *D. oregonensis* males and *D. minutus* females by size-ratio, and plotted relative frequency of attempts against respective ratios (Fig. 5). Despite the overall negative relationship between variables ($r = -0.14$), the large scatter in data rendered the relationship statistically non-significant ($P > 0.5$). Even when we sorted the data by lake, we found no significant relationship between relative frequency and sex-size-ratio for sympatric populations.

With few exceptions, heterospecific pairing of *D. minutus* males with *D. oregonensis* females did not result in spermatophore transfer; only five of 125 males attempted to mate. There was also no significant effect of reproductive condition or size-ratio on the mating data. These observations indicate that *D. minutus* males cannot or do not engage in heterospecific copulation to the point where a spermatophore is transferred.

Mating in Epischura

The reproductive biology of *Epischura* is fundamentally different from that of *Diaptomus*. First, unlike *Diaptomus*, which usually detaches spent spermatophores before extrusion of a clutch, *Epischura* retains it for a considerable period, even while a clutch is being extruded. Secondly, whereas *Diaptomus* extrudes the eggs into a sac, bears the clutch for 1–3 days, and then sheds it as a unit, *Epischura* extrudes eggs individually, and releases them immediately without bearing the clutch in a sac. If eggs are borne in a clutch, the clutch breaks apart relatively quickly. This has the effect of scattering eggs over a considerable distance, especially if the female is swimming during the process. The third major difference is that diaptomid eggs are brooded in the egg sac, whereas eggs of *Epischura* normally develop in the water column or sediment, away from the female. Egg development in *Epischura* requires 3 or 4 days at 20°C in the laboratory.

TABLE 3. Mean clutch size of *Epischura* females. Clutch order refers to order in which multiple clutches were extruded. n = sample size.

Month	Clutch order	Clutch size	n	SE
June	First	16.21	33	0.76
	Second	10.84	19	1.41
	Third	5.55	11	0.99
	Fourth	6.00	2	—
August	First	24.07	14	1.82
	Second	11.40	5	2.01

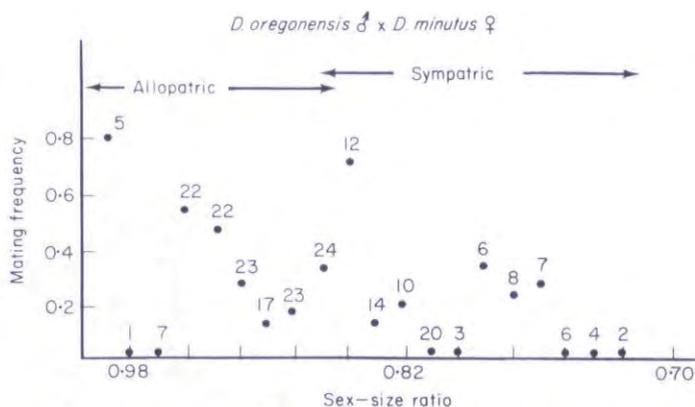


FIG. 5. Frequency of mating attempts plotted as a function of corresponding size-ratio. Arrows indicate the range in ratios that correspond to respective experiments involving allopatric and sympatric populations. Numbers in the body of the figure as in Fig. 4.

Lastly, *Diaptomus* only produces one clutch per mating; *Epischura*, on the other hand, can produce up to four clutches from one spermatophore attachment (Table 3).

Given that female *Epischura* can produce multiple clutches from a single mating, two new categories were examined: females that had previously mated, and those that had not (virgins). Females in this latter category were newly moulted females 1–2 days old. Those in the first category included females that had retained their spent spermatophores and a few whose spermatophores had become dislodged from their genital pores.

Males in these experiments only attempted to mate with virgin females (Fig. 6); however, ovary development in these virgins did not have any significant effect on mating frequency. There was a significant effect of size-ratio ($\chi^2=14.59$, $df=6$; $P<0.025$) since mating attempts were recorded exclusively for experiments with corresponding sex-size-ratios between 1.08 and 1.14. Because relative frequencies were all 1.0, it was not possible to determine peak frequency.

Life history and reproductive characteristics

The mean life span of *D. minutus* females collected from Trouser and Sally Lakes were 39.0 and 41.7 days, respectively (Fig. 7a). These

values are similar to those of *D. oregonensis* from Trouser, Nick and Moulin Lakes (30.6, 34.0 and 44.4 days, respectively). A two-way analysis of variance (based on unequal sample size) revealed no statistical differences in mean female lifespan from lake to lake, or from species to species ($P>0.05$). The lifespan of diaptomid males was generally less than that of females within the same lake. There were insufficient data from Nick and Trouser Lakes to facilitate statistical analysis, but an analysis of variance for remaining data indicated that, regardless of species, mean lifespan of males did not differ among lakes ($P>0.05$).

Proportion of females' adulthood spent in the gravid condition was computed for both species. *D. minutus* females spent 55–60% of their adulthood in the gravid condition, whereas *D. oregonensis* only spent 20–30% of their adulthood in this condition (Fig. 7b). The interval between gravid periods for *D. minutus* was also significantly reduced compared with that for *D. oregonensis* ($P<0.05$), even though there was great among-lake variation within species (Fig. 7c).

The number of days between final moult and the initial gravid period also differed significantly between species (Fig. 7d) under our controlled laboratory conditions; that for *D. minutus* ranged from 2 to 3 days, while that for *D. oregonensis* ranged from 4.5 to 6 days. The mean

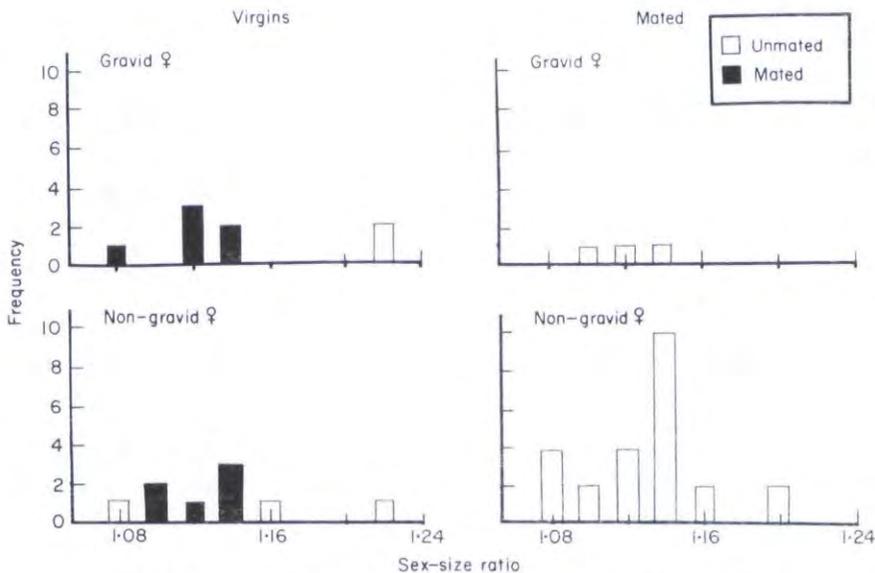


FIG. 6. Frequency of mating attempts for corresponding size-ratios.

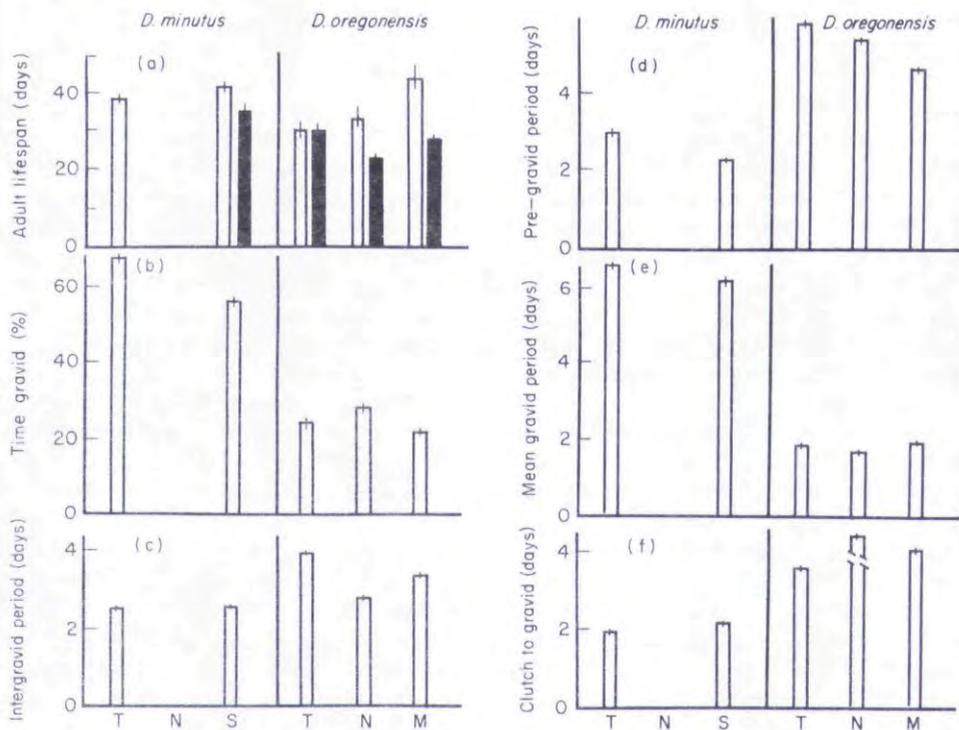


FIG. 7. Summary of reproductive and life history characteristics for diaptomid species: (a) mean ± 1 SE adulthood of males (striped) and females (open); (b) mean ± 1 SE proportion of female adulthood spent in gravid condition; (c) mean ± 1 SE interval between gravid periods; (d) mean ± 1 SE interval between final moult and initial gravids period; (e) mean ± 1 SE duration of gravid period; (f) mean ± 1 SE duration between clutch formation and onset of gravid condition (duration in days). T=Trouser Lake; N=Nick Lake; S=Sally Lake; M=Moulin Lake.

duration of the gravid period for *D. minutus* females was more than 3 times higher than that for *D. oregonensis* females (Fig. 7e). We also calculated the mean duration between clutch formation (when the female carried one at the start of the experiment) and onset of the next gravid period. *D. oregonensis* females in general required longer than 3.5 days to accomplish this, whereas *D. minutus* required less than 2.5 days (Fig. 7f).

Results of these experiments indicated that reproductive characteristics of these two diaptomids are species-specific, and for the most part are unaffected by lake origin. Since conditions in these experiments were identical, differences between species cannot be attributed to environmental factors such as food, temperature or presence/absence of mates, unless species respond differently to these factors.

Copepods varied greatly with respect to clutch size from population to population in the field (Fig. 8). A two-way analysis of variance showed

that lake origin and month of year had significant effects on clutch size ($P < 0.05$). This is not unexpected since clutch size is directly related to temperature and food conditions (Elmore, 1983), and these fluctuate greatly from May to August in lakes. The most notable trend is that clutch size of *D. oregonensis* was generally larger than that of *D. minutus*, except in August, at which time only animals in Trouser Lake bore large clutches.

Discussion

We can now review relevant information to evaluate applicability of some of the proposed mechanisms for reproductive isolation. Since there was no apparent temporal partitioning (Fig. 1), no apparent divergence in onset of maturity (Fig. 2), and no summer seasonality in laboratory experiments with respect to mating frequency (Fig. 3) for the two diaptomid species,

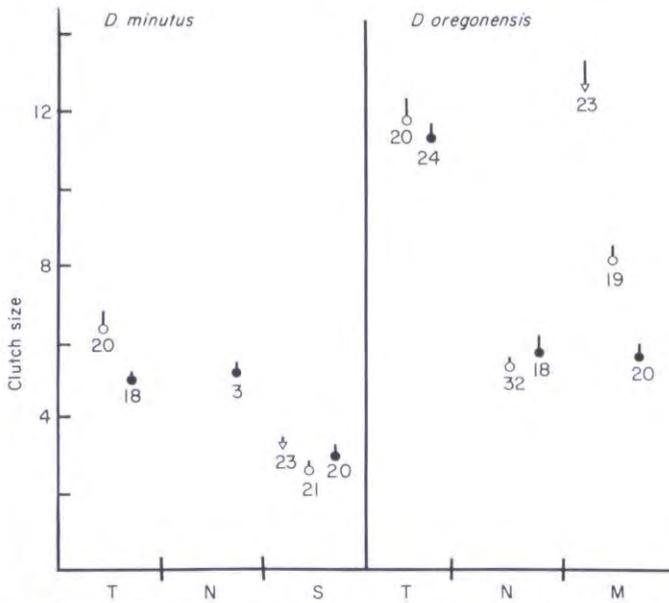


FIG. 8 Summary of mean (± 1 SE) clutch size of diaptomids. T=Trouser Lake; N=Nick Lake; S=Sally Lake; M=Moulin Lake. ∇ , June; \circ , July; \bullet , August.

we can eliminate divergence in timing of reproductive events as a possible mechanism.

We also found no evidence that divergence in size (Maly, 1984) prevented interbreeding between sympatric diaptomid populations. Incidence of heterospecific mating in organisms taken from Trouser Lake was nearly as high as that noted for organisms taken where they occur allopatrically, even though corresponding size-ratios in the former were substantially larger (Fig. 5). Thus, size displacement between sympatric populations was not accompanied by a significant concomitant reduction in heterospecific mating.

By the process of elimination, the mechanism that probably applies here is divergence in behaviour associated with development of species-specific cues for preferential detection of gravid females or species-specific receptivity of gravid females for mating partners. Several observations lend credence to this. The fact that males mated discriminantly with conspecific gravid females, but did not distinguish gravid from non-gravid heterospecific females, implies that the major attraction is species-specific (Table 1; Figs. 4 and 5). We also know that cues responsible for this initial attraction are probably chemosensory (Kittredge *et al.*, 1974; Katona, 1973, 1975) rather than morphological in nature

since non-gravid females were usually ignored even though gravid females of similar size were mated. We therefore support speculations made by many others that sex pheromone is the primary male attraction in mating of calanoid copepods. We further speculate that production, release and detection of this pheromone is species-specific, and thus functions as a mechanism for reproductive isolation in *Diaptomus*.

We cannot, however, preclude the possibility that gravid females are also exhibiting pre-copulatory behaviours to attract males, which are therefore more receptive to mating than non-gravid females.

The advantage in short-circuiting a potentially unsuccessful copulation at the 'attraction' phase is that males do not waste gametes, and do not spend unnecessary energy in pursuit of non-receptive conspecific, or heterospecific females; at the same time, females also benefit from this since they do not waste their gametes (transfer of heterospecific spermatophores results in extrusion of non-viable clutches (Jacoby & Youngbluth, 1983)).

There also appears to be an interactive effect of reproductive condition and size-ratio on mating frequency of *D. minutus* (Fig. 4b); peak in mating activity associated with size-ratio was only observed when gravid females were consid-

ered. The ratio associated with this peak falls within 1.10 and 1.12, bracketing the observed 'optimum' size-ratio of 1.10 that corresponds to peak mating intensity in field populations of *D. minutus* observed by DeFrenza *et al.* (1986). Thus, data collected in these relatively simple laboratory trials reflect behaviour exhibited by natural diaptomid communities. By comparison, the interactive effect between reproductive condition and size-ratio for *D. oregonensis* was more difficult to ascertain since no distinct peak in mating frequency was evident (Fig. 4d), but the range of sex-size-ratios tested was not as broad as that examined by DeFrenza *et al.* (1986).

Results from mating experiments with *Epischura* point to a somewhat different scenario (Fig. 6). Since *Epischura* did not discriminate between gravid and non-gravid females, release of pheromones, if produced by this species, is probably independent of ovary development, and most likely commences soon after the final moult. Correct placement of a spermatophore on the female's genital pore may inhibit further production and release of this pheromone (Hopkins & Mackin, 1977), since males did not attempt to mate with females that had spermatophores attached to them from a previous mating. The fact that males did not copulate with previously mated females, even when spermatophores were subsequently dislodged, also implies that production and release of pheromone do not re-occur once the process has been arrested.

That sex-size-ratio should have an effect on frequency of mating attempts for *D. minutus* (Fig. 4) and *Epischura* (Fig. 6) suggests that morphological cues may also function in mate recognition more strongly in these species than in *D. oregonensis*, assuming that cues operate after initial contact has been made. *Epischura* may have evolved mate recognition based on relative size because there is virtually no other large calanoid present in the lake to interfere with successful copulation at the time when *Epischura* engages in peak mating activity (Fig. 1), at least near Montreal. In such a situation, any female of appropriate size may be mated with no attending ill consequences. This behaviour may also capitalize on the fact that females apparently only mate once (Fig. 6), so that males who wait until females become gravid are disadvantaged.

The reason for *D. minutus*' apparent strong reliance on morphological cues is more complicated to explain and may involve reproductive and life history characteristics of both diaptomid species. Adult females of both species have statistically similar survivorship when cultured in the laboratory (Fig. 7a). Under controlled conditions, the main difference between species is that females of *D. minutus* spend on the average 60% of their adulthood in the gravid condition, whereas *D. oregonensis* spend approximately 30% of their adulthood in this condition (Fig. 7b-f).

Given that gravid condition is prerequisite to successful mating, the fact that *D. minutus* females are gravid much more often than are *D. oregonensis* implies that the former should be more 'available' for mating. Since males of *D. minutus* may be less constrained by availability of receptive females, the added criterion they use to select suitable mates, is an asset rather than a liability, as supported by the low incidence of heterospecific mating between *D. minutus* males and *D. oregonensis* females. By contrast, *D. oregonensis* females are gravid only a small proportion of their adulthood, so that males must take full advantage of times when females are gravid. By virtue of their poorer discrimination, however, males engaged in heterospecific mating with *D. minutus* females at a higher frequency (Fig. 5), and thereby wasted many more gametes.

The reproductive strategies for these calanoid copepods appear to be a trade-off among the following: (1) degree of discrimination required for mate selection, (2) availability of mates, and (3) clutch size. In *D. minutus*, clutch size of females varies from two to seven eggs (Fig. 8), and males appear to be extremely selective, probably relying on both chemical and morphological cues for mate recognition, however, females compensate for small clutch size by being frequently available to males. In *D. oregonensis*, females are less frequently available for mating, but males appear to be less discriminating in choice of mates. Females, however, compensate for less frequent reproduction by producing considerably larger clutches (6-13 eggs/clutch, Fig. 8). In *Epischura*, females appear to be available for an extremely short time period, and males mate only with virgin females; females, however, bear multiple clutches from a single mating (6-23 eggs/clutch, Table 3).

Whereas sex pheromones are probably involved in distance location of females by males, behavioural and morphological cues are probably more important for post-contact recognition. This is supported by our observation that size-ratio had a significant effect on mating frequency in mating trials involving gravid females (Fig. 4). Future experiments should be conducted to determine if the sex pheromone is involved in mate location and mate recognition.

Diaptomid species are often found sympatrically in many North American lakes and ponds. The ability to copulate discriminantly with reproductively compatible mates probably helps to perpetuate a successful coexistence in spite of their ecological similarity. We hope that this study will further stimulate investigators to identify the sex pheromone that may be responsible for mate attraction and recognition, and to ascertain the species-specific nature of this pheromone.

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