Sexual compatibility between two heterochronic morphs in the Alpine newt, *Triturus alpestris*

MATHIEU DENOËL, PASCALPONCIN & JEAN-CLAUDE RUWET

*Laboratory of Fish and Amphibian Ethology,
Department of Ethology, University of Liège*

Abstract

Paedomorphosis, in which individuals retain ancestral characteristics in the adult stage, is widespread in newts and salamanders and is suspected to play an important role in evolution. In some species, paedomorphosis is facultative with some individuals forgoing metamorphosis. Optimality models have been proposed to explain the maintenance of this polymorphism, but require the integration of reproductive patterns into the models. We investigated the frequencies of inbreeding and outbreeding in two syntopic heterochronic morphs of the Alpine newt. The two morphs are sexually compatible: encounters between and within morphs were equally successful in terms of spermatophore transfer. Behavioural observations were in agreement with the sexual compatibility observed. Nevertheless, paedomorphic males displayed to females less frequently than metamorphic males. The two morphs differ largely on the basis of sexual secondary characteristics, but the majority of these traits did not affect mating success. Because of the large flow of genes between the two heterochronic morphs and because of the absence of spatial and temporal isolation, these results do not support sympatric speciation models, but are in favour of the maintenance of polymorphism in natural populations.

© 2001 The Association for the Study of Animal Behaviour

Introduction

Sexual isolation is the tendency of individual organisms to avoid mating with those of another strain, race, or species (Gilbert & Starmer 1985). Numerous studies have examined sexual isolation between species, subspecies and populations (Verrell & Tilley 1992; Hollocher et al. 1997). Some have revealed asymmetries in sexual isolation (Arnold et al. 1996; Michalak et al. 1997) and others have provided a behavioural basis for it (Hoikkala 1986; Michalak et al. 1997).

Paedomorphosis is a heterochronic process in which juvenile characters are retained by the adults (Gould 1977). It is particularly obvious in newts and salamanders because it implies sexual maturation before metamorphosis (Whiteman 1994). In some species, the two ontogenetic pathways (i.e. paedomorphosis and metamorphosis) are present in the same population. Both genetics (Tomkins 1978; Voss & Shaffer 1997) and specific environmental factors (Harris 1987; Semlitsch 1987) are involved. Permanent waters and low densities induce larvae to opt for a paedomorphic ontogenetic pathway (Harris 1987; Semlitsch 1987). A single gene was once considered to underlie the life history pathway differences (Tompkins 1978), but the situation is now known to be more complex (Voss 1995).

Paedomorphs and metamorphs differ largely in morphology. Paedomorphs retain external gills, whereas metamorphs are fully metamorphosed (Semlitsch & Wilbur 1989). In addition, the two morphs present different epigamic characters (Bovero 1991). Sexual selection theory (Darwin 1871; Andersson 1994) predicts that females may choose between males on the basis of their secondary sexual characters. This was shown to be the case in metamorphic newts (Hedlund 1990; Green 1991; Gabor & Halliday 1997). Because paedomorphs have a larval structure, their swimming (Shaffer et al. 1991) and aerobic capacity (Seliskar & Pehani 1935) may also affect their sexual performance.

Although it is known that paedomorphs can reproduce together and interbreed with metamorphs (Semlitsch & Wilbur 1989; Bovero 1991; Krenz & Sever 1995; Whiteman et al. 1999), the success rates of homotypic and heterotypic sexual encounters remain unknown. In other groups, such as fish and birds, sexual isolation between morphs is also poorly documented (Maekawa & Onozato 1986; Smith 1990; Ziuganov & Zotin 1995) and, to our knowledge, no studies have addressed the question of behavioural asymmetry between such morphs.

The maintenance of facultative paedomorphosis can be favoured by an earlier age at maturity (Denoël & Joly 2000; Ryan & Semlitsch 1998), resource partitioning (Whiteman et al. 1996; Denoël & Joly, in press) and breeding frequency (Whiteman 1997).

Correspondence: M. Denoël, Laboratory of Fish and Amphibian Ethology, Department of Ethology and Animal Psychology, Institute of Zoology, University of Liège, Quai Van Beneden 22, 4020 Liège, Belgium (email: Mathieu.Denoel[a]ulg.ac.be).

But we do not know how sexual selection can influence it (Whiteman et al. 1999). Indeed, whereas sexual isolation may be the first step in the formation of two species in sympatry (Smith & Skulason 1996; Seehausen et al. 1999), sexual compatibility may promote the maintenance of polymorphism by ensuring gene flow between morphs (Kornfield et al. 1982; Roff & Fairbairn 1991).

We investigated the question of sexual isolation and behavioural asymmetry between syntopic heterochronic phenotypes. We also present data on morphological characteristics of males and those stages of courtship thought to be important in allowing sexual isolation.

METHODS

Study Species

The Alpine newt is an amphibiontic species widely distributed in Europe. Sexual behaviour occurs in the aquatic habitat. There are three main phases. First, the male meets the female and may follow her if she moves away (orientation phase). Then, the male tries to adopt a static posture in front of the female and performs several displays, such as the fan, a rapid tail vibration movement (display phase). Finally, the male may deposit a spermatophore on the substratum which the female may or may not pick up (spermatophore transfer phase; Halliday 1977; Denoël et al. 2001). In our experimental set-up, we defined a 'responsive' female as one that moved towards the male or turned her head towards his tail or head, and we called these responses 'positive'; we defined an 'unresponsive' female as one that remained stationary or moved away from the male, and we called these responses 'negative' (Denoël et al. 2001).

We collected adult Alpine newts from an Alpine lake, Lac de la Cabane (Alpes-de-Haute-Provence, France, UTM: 32TKQ91), the week after the lake thawed, at the start of the breeding season. To catch the newts, we used a landing net from the shore. The first group was caught in May 1998 (paedomorphs: 32 males and 36 females; metamorphs: 28 males and 24 females) and the second in May 1999 (paedomorphs: 33 males and 42 females; metamorphs: 20 males and 16 females). We kept the newts in refrigerated boxes (5–10 C; 30 x 20 cm and 12 cm high) to transport them to the laboratory. Then, sexes and morphs were kept separately in seven aquaria (four of 250 x 40 cm and 40 cm high, two of 100 x 100 cm and 40 cm high, and one of 200 x 100 cm and 40 cm high). They were fed four times a week with *Chironomus* larvae and *Daphnia*. Temperature ranged between 12.5 and 14.5 C and the photoperiod was 1400 hours light/0800 hours dark. These laboratory conditions are within the range met by the newts in the field. All the newts were released in good health to their native lake after the experiments: May 1999 for the first group and May 2000 for the second.

Adulthood was established on the basis of a well-developed cloaca, and sex on the presence of secondary sexual characters (e.g. dorsal crest, colour, shape of the cloaca). An individual was considered to be a paedomorph if the gill slits were open and a metamorph if they were closed, that is, if it was metamorphosed.

The research was authorized by the French Environment Ministry.

Observation Protocol

We staged 248 encounters involving the four possible crosses: male paedomorph and female paedomorph (PP = 99); male metamorph and female metamorph (MM = 59); male paedomorph and female metamorph (PM = 46); and male metamorph and female paedomorph (MP = 44). Individuals were randomly assigned to their sexual partners. All encounters were unique as no individual males and females encountered one another more than once.

In each experiment, we placed a male and a female, both randomly chosen, together in an aquarium (45 x 30 cm and 25 cm high). Sexual interactions were recorded with a Sony Hi8 camcorder. Because large variations in temperature can influence newt courtship (Denoël 1998), we maintained water temperature at a mean ± SD of 14.0 ± 0.5 C. Light intensity was 5000 lx on the bottom of the aquarium from two neon tubes at the top. If the male did not display during the first 45 min of the experiment, both individuals were replaced by two new ones. Otherwise, we stopped experiments when sexual behaviour had finished (i.e. no directed sexual behaviours from one individual towards the other had occurred for at least 1 min). We renewed the water after each experiment to eliminate pheromones. Viewing the tapes enabled us to time the succession of male and female behaviour on a computer. After the experiment, and to identify individuals, we toe-clipped the newts with a code similar to that of Twitty (1966), but we did not cut more than two toes of any one newt. Toe clipping does not affect survival and body condition of *Triturus* newts (Arntzen et al. 1999) and toes are known to regenerate in less than 1 year (Gütleb 1991). Because we used a large number of individuals, it was not possible to avoid a marking technique. Toe clipping appears to be the best method to recognize newts in the laboratory: tattooing would interfere with secondary sexual characteristics and pit tags would damage the skin. At least 2 days were allowed after

Table 1. Mating frequency and coefficients of isolation asymmetry, propensity asymmetry and joint sexual isolation for four stages of courtship in sexual encounters between paedomorphs (P) and metamorphs (M)

| Phase | Mating frequency | | | | Isolation asymmetry | Propensity asymmetry | Joint sexual isolation |
	π_{PP}	π_{MM}	π_{PM}	π_{MP}			
Displaying males	0.500 (24)	0.813 (16)	0.667 (18)	0.640 (25)	0.027 ± 0.147	0.313+-0.141*	0.006±0.204
Success of encounters	0.125 (24)	0.250 (16)	0.111 (18)	0.160 (25)	0.049 ± 0.104	0.125 ± 0.128	0.104 ± 0.165
Success of displaying males	0.211 (19)	0.308 (13)	0.133 (15)	0.235 (17)	0.102 ± 0.135	0.097 ± 0.159	0.150 ± 0.208
Success of males transferring spermatophores	0.278 (18)	0.333 (12)	0.143 (14)	0.132 (14)	0.286 ± 0.162	0.056 ± 0.172	0.040 ± 0.236

Means are given ± SE. Sample sizes are given in parentheses. For definitions see Methods.
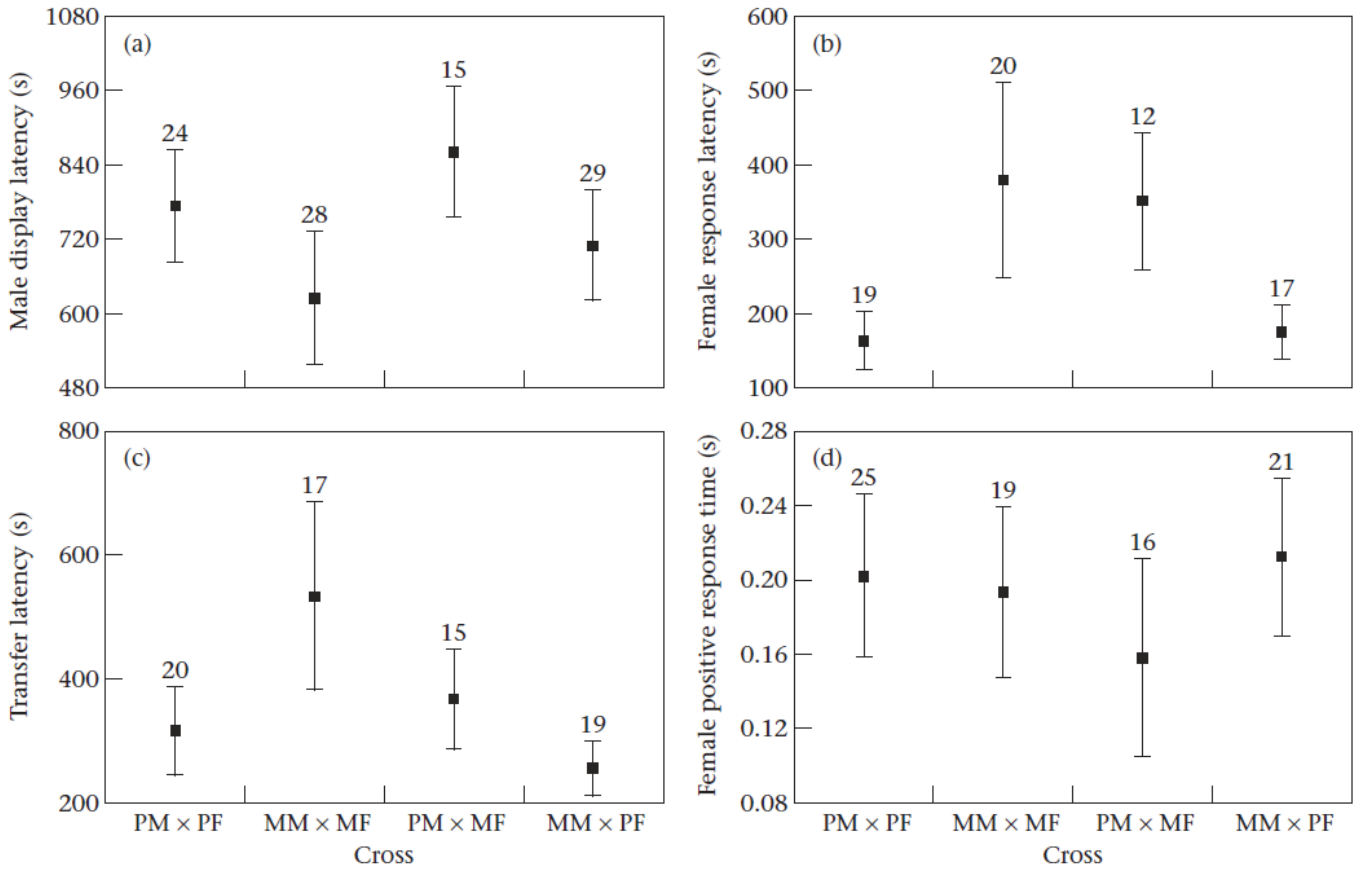*$P < 0.05$.

Figure 1. Mean duration ± SE of (a) male display latency (time from the entrance of both individuals in the aquarium to the first display of the male to the female), (b) female response latency (time from the start of the male displays to the first positive response of the female, i.e. movement towards the male), (c) transfer latency (time from the first displays to the first spermatophore deposition) and (d) female positive response time (proportion of time during which female was responsive to the male) for the four crosses: male paedomorph x female paedomorph (PM x PF), male metamorph x female metamorph (MM x MF), male paedomorph x female metamorph (PM x MF) and male metamorph x female paedomorph (MM x PF).

toe clipping before behaviour patterns were observed. Observations took place in the 40 days after the capture date. This period does not exceed the breeding period in natural populations (e.g. Cei 1943). We measured the following after each experiment: in males, snout–vent length, body mass, tail depth, cloaca width, number of dots on the flanks; in females, snout–vent length and body mass. Lengths were taken with a precision of 1 mm and mass with 0.1 g.

We recorded four categories of mating frequencies: displaying males (proportion of males that display); success of encounters (proportion of successful mating); success of displaying males (proportion of displaying males that were successful in sperm transfer); and success of transferring males (proportion of males depositing sperm that were successful in mating). An event was called successful when the female picked up at least one spermatophore.

We measured the following behaviour patterns: male display latency (s) from the entrance of both individuals in the aquarium to the first display of the male to the female; female response latency (s) from the start of the male displays to the female's first positive response; transfer latency (s) from the first display to the first spermatophore deposition; and female positive response time (proportion of time during which the female was responsive to the male). We consider these behaviours to be important in determining sexual isolation between individuals. Crosses involving compatible individuals would have a shorter male display latency, female

response latency and transfer latency and a higher female positive response time than crosses involving incompatible individuals.

Statistical Analyses

The probabilities of success (spermatophore transfer) were determined for the four kinds of crosses (PP, MM, PM, MP). They were then used to calculate measures of isolation asymmetry, propensity asymmetry and joint isolation.

Isolation asymmetry, IA (Arnold et al. 1996; Michalak et al. 1997), measures the discrepancy in incidence of mating between the two types of heterotypic pairings and ranges from 0 (no asymmetry) to 1 (complete asymmetry),

$$ IA = \mid MP - PM \mid . $$

Propensity asymmetry, PA (Arnold et al. 1996; Michalak et al. 1997), measures the discrepancy in incidence of mating between the two types of homotypic pairings and ranges from 0 (no asymmetry) to 1 (complete asymmetry),

$$ PA = \mid MM - PP \mid . $$

Joint isolation, JI (Verrell & Tilley 1992; Arnold et al. 1996; Michalak et al. 1997), measures the overall breakdown in mating when pairings are staged between partners from different morphs and ranges from 0 (when within-morph [homotypic] and between-morph [heterotypic] proportions are equal) to 2 (when all homotypic but no heterotypic encounters are successful),

$$JI = MM + PP - MP - PM.$$

Standard errors of IA, PA and JI were calculated according to McCullagh & Nelder (1989) and Arnold et al. (1996). We tested the null hypothesis that each coefficient was equal to zero by using SEs to calculate values of the ts statistic. The values were then tested against the t distribution.

Because we used some individuals more than once, we averaged values on each individual before computing the statistical tests. Time and morphological data were normalized by arcsine and log transformation (for proportions and counts, respectively) before we calculated the parametric significance tests. Because some traits vary with snout–vent length, an analysis of covariance was performed. Since it is not possible to correct standard errors of coefficients of isolation and asymmetry with respect to unequal repeated measurements, we considered only the independent encounters for the analysis (and omitted encounters involving an individual previously used). Consequently, each individual was used only once for these isolation and asymmetry analyses.

All statistical tests are two tailed.

RESULTS

Table 1 shows the probabilities of success of the four kinds of crosses (PP, MM, PM, MP) and coefficients of isolation asymmetry, propensity asymmetry and joint isolation. Propensity asymmetry was significant for the proportion of displaying males, homotypic encounters involving paedomorphs being less successful than those involving metamorphs. No other coefficient was significant (Table 1).

There was no significant difference between the four crosses for male display latency (ANOVA: $F_{3,92}$ = 1.602, P = 0.19; Fig. 1a), female response latency (ANOVA: $F_{3,64}$ = 0.955, P = 0.42; Fig. 1b), transfer latency (ANOVA: $F_{3,67}$ = 1.300, P = 0.28; Fig. 1c) and female positive response time (ANOVA: $F_{3,77}$ = 0.446, P = 0.72; Fig. 1d).

Male metamorphs (N = 34) and paedomorphs (N = 45) differed significantly for all measures taken except snout–vent length (t test: t_{77} = 1.346, P = 0.18). Scores were higher in metamorphs than in paedomorphs for number of dots on the flanks (ANCOVA: $F_{1,76}$ = 58.841, P < 0.001; Fig. 2a) and width of cloaca (ANCOVA: $F_{1,76}$ = 13.651, P < 0.001; Fig. 2c) and lower in metamorphs than in paedomorphs for tail depth (ANCOVA: $F_{1,76}$ = 91.686, P < 0.001; Fig. 2b) and body mass (ANCOVA: $F_{1,76}$ = 17.188, P < 0.001; Fig. 2d).

Table 2 shows the correlation coefficients determining the relation between male characters and female positive response time, female response latency and encounter success. The significance of correlations was tested with a t test. The majority of the correlations are not significant, except cloaca with paedomorphic female positive response time.
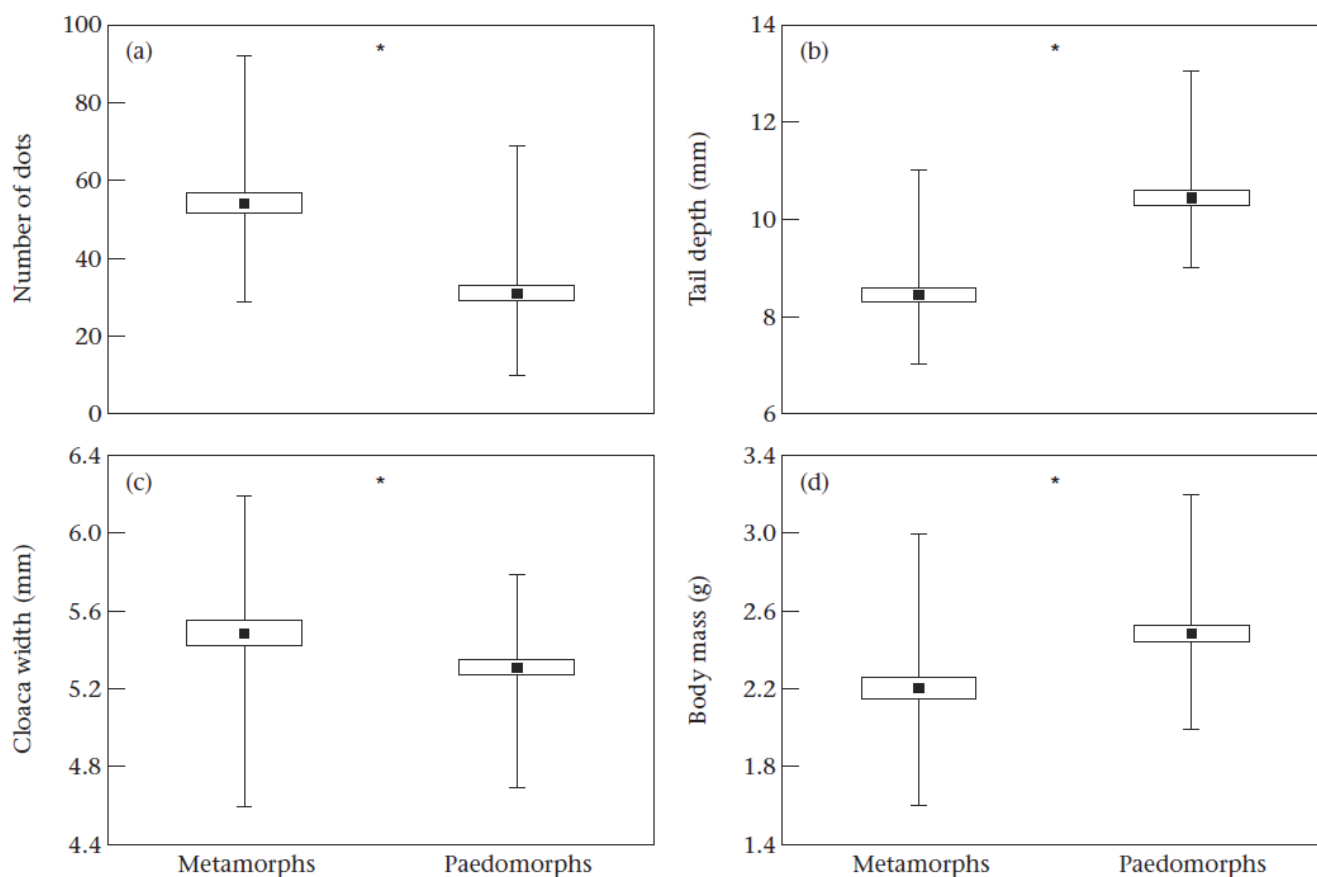

Figure 2. Morphological characters of male metamorphs (N = 34) and paedomorphs (N = 45). (a) Number of dots on both flanks (b) tail depth, (c) cloaca width and (d) body mass. Point, mean; box, SE; whiskers, range. *P < 0.001 (ANCOVA).

Table 2. Coefficients of correlation between morphological characters of males and three measures of sexual encounter success in female metamorphs and paedomorphs: female positive response time (time during which females were responsive), female response latency (time elapsed from the first male display to the female's first positive response) and encounter success (considering only encounters during which males displayed

Female response	Male character	Female metamorphs				Female paedomorphs			
		N	r	$t_{(n-2)}$	P	N	r	$t_{(N-2)}$	P
Female positive response time	dots	35	0.129	0.745	0.46	46	0.055	0.366	0.72
	cloaca	35	0.015	0.087	0.93	46	0.318	2.225	0.03
	tail depth	35	-0.179	-1.043	0.31	46	-0.163	-1.097	0.28
	length	35	-0.092	-0.531	0.60	46	0.066	0.44	0.66
	body mass	35	0.068	0.395	0.70	46	-0.005	-0.034	0.97
Female response latency	dots	32	0.077	0.424	0.68	36	-0.062	-0.364	0.72
	cloaca	32	-0.031	-0.170	0.87	36	-0.290	-1.765	0.09
	tail depth	32	-0.229	-1.290	0.21	36	0.177	1.046	0.30
	length	32	0.208	1.164	0.254	36	-0.048	-0.280	0.78
	body mass	32	-0.064	-0.350	0.73	36	0.014	0.084	0.93
Encounter success	dots	36	0.225	1.346	0.19	52	0.035	0.248	0.805
	cloaca	36	-0.088	-0.512	0.61	52	0.126	0.898	0.37
	tail depth	36	-0.301	-1.842	0.07	52	-0.054	-0.381	0.71
	length	36	-0.056	-0.327	0.75	52	-0.099	-0.702	0.49
	body mass	36	0.003	0.016	0.99	52	-0.145	-1.039	0.30

Data were normalized by arcsine (female positive response time) or log (female response latency and encounter success) transformation before we did the tests.
Length = snout–vent length.

DISCUSSION

Our results reveal that the two heterochronic morphs we studied are sexually compatible. Success rate was 21% in homotypic encounters and 16% in heterotypic encounters; the number of spermatophores picked up by the female was 11% in homotypic encounters and 10% in heterotypic encounters. Similar success during heterotypic and homotypic encounters resulted in a coefficient of joint isolation not different from zero. This is contrary to studies dealing with different populations or species of newts and salamanders (Arnold et al. 1996; Michalak et al. 1997). It thus appears that whatever their ontogenetic pathway, newts of one morph can breed with individuals of the other. However, a propensity asymmetry was revealed. Indeed encounters involving paedomorphs appeared less successful (13%) than those involving metamorphic individuals (25%). Paedomorphic males thus displayed to females less than metamorphs.

The behavioural parameters (male display latency, female response latency, transfer latency and female positive response time) we analysed are all consistent with sexual compatibility between the two morphs, indicating that neither male nor female showed directional choice for either morph. Although high competition resulting from a biased operational sex ratio may also favour shorter courtship latencies, it would do so in both compatible and incompatible individuals. The behavioural measures are thus cues to the sexual compatibility between the two morphs.

The males of the two morphs differ considerably in morphology. While metamorphs have a large cloaca and numerous dots, paedomorphs are heavy with deep tails. On the basis of sexual selection theories

(Darwin 1871; Arnold 1983; Andersson 1994), we should expect males with conspicuous sexual secondary characteristics to be more successful. That was shown to be the case in several newt species (Hedlund 1990; Green 1991; Gabor & Halliday 1997), but in our experiments, the majority of the characteristics analysed did not appear to be linked to success or to female responsiveness. Only the cloaca was significantly linked to female responsiveness, but this correlation may be significant by chance given the large number of correlations performed. This absence of selection may be due in part to a low selectivity at the start of the reproductive period, when we did our study. It was effectively shown in *T. vulgaris* that females become more selective after being inseminated (Gabor & Halliday 1997). Nevertheless, even if females become choosier later in the season, the two morphs clearly breed together when they are most ready to get mates. Future studies involving fertilized females are thus needed to model the influence of operational sex ratio and individual experiences on the outcome of sexual interactions between heterotypic morphs. Michalak & Rafinski (1999) and Verrell (1990) also pointed out that discrimination might occur mainly in experiments in which females were given a choice. Experiments involving three individuals will tell us more about the effect of choice on the sexual isolation of two heterochronic morphs, but preliminary data reveal that females are responsive to both kinds of males in triadic encounters (M. Denoël, unpublished data). It would also be interesting to record the response of a female confronted with successive males as well as with progenetic males (i.e. paedomorphic males reaching sexual maturity earlier than metamorphic males) which occurs in some populations (Denoël & Joly 2000). Although largely dimorphic in morphology, it is possible that the two morphs produce similar courtship pheromones. Because such chemi-

cals can also attract females (Kikuyama et al. 1995), their similarity could favour sexual compatibility between morphs.

In a population of *Ambystoma talpoideum*, Krenz & Sever (1995) showed that sexual isolation might occur because paedomorphs breed earlier than metamorphs and so paedomorphic males appear to mate primarily with paedomorphic females. Such assortative mating could make offspring of paedomorphs less likely to metamorphose (Semlitsch & Wilbur 1989). In our population, this is not the case. Indeed, the majority of the metamorphs remained under water the whole year. We observed them in the lake from the thawing to the freezing of the water and no individual was seen to migrate on land to reach it. The two morphs breed at the same time, just after the lake thaws, and we did not find any temporal isolation between them. They are not explosive breeders. At the time we caught them in the lake, they had just started to court. Indeed, temperatures were still very low during the first week after the lake thawed (maximum 5–8 C; M. Denoël, unpublished data). The initiation of sympatric speciation has been proposed through habitat shift (Smith & Skulason 1996). However, the two heterochronic morphs of the Alpine newt breed within the same habitat in this lake (M. Denoël, unpublished data).

Whiteman (1994) provided several hypotheses to explain the maintenance of facultative paedomorphosis. With respect to mating success, his 'paedomorph advantage hypothesis' predicts a mating advantage for male paedomorphs, while his 'best of a bad lot hypothesis' predicts decreased mating success in male paedomorphs. These predictions involve body size, which would be linked to competitive ability. From our data, both morphs have access to reproduction, but paedomorphs seem a little disadvantaged, as they were less successful than metamorphs. This was not due to size differences, but rather to male mating activity. Although morphologically adult, not all of the individuals showed sexual activity. It thus appears that there is a cost to adopting a paedomorphic ontogenetic pathway. Hollocher et al. (1997) considered that, everything else being equal, the mating advantage of a particular type over another type should have driven the advantaged genes to fixation. The answer to why the polymorphism is maintained would be found in specific advantages of the other type. In our newt population, the age structures of the two morphs are similar so there is no paedomorph advantage for that life history trait (Denoël & Joly 2000), but their fecundity is still unknown. On the other hand, in La Cabane Lake, the paedomorphic tactic appears adaptive in allowing resource partitioning (Denoël & Joly, in press). The advantage gained by paedomorphs may then be selected as they can breed together and with metamorphs. On the other hand, maintenance of metamorphs in the lake could be favoured by a better mating success (this study), but also by feeding advantages (M. Denoël, unpublished data) and by regular catastrophes such as drying up (Denoël & Joly 2000), which induces metamorphosis of paedomorphs (Semlitsch 1987).

The absence of selection on the male characters may favour polymorphism. This was shown in trophic morphs of cichlid fish (Kornfield et al. 1982) and in migratory morphs of insects (Roff & Fairbairn 1991). High sexual compatibility between heterochronic morphs allows an unrestricted gene flow between sympatric morphs. Consequently, our results do not support models of sympatric speciation that consider polymorphisms as an intermediary step to sympatric speciation (Smith & Skula-son 1996; Seehausen et al. 1999), but rather are in favour of the maintenance of facultative paedomorphosis as a valuable option.

References

Andersson, M. 1994. Sexual Selection. Princeton, New Jersey: Princeton University Press.

Arnold, S. J. 1983. Sexual selection: the interface of theory and empiricism. In: Mate Choice (Ed. by P. Bateson), pp. 67–107. Cambridge: Cambridge University Press.

Arnold, S. J., Verrell, P. A. & Tilley, S. G. 1996. The evolution of asymmetry in sexual isolation: a model and a test case. Evolution, 50, 1024–1033.

Arntzen, J. W., Smithson, A. & Oldham, R. S. 1999. Marking and tissue sampling effects on body condition and survival in the newt *Triturus cristatus*. Journal of Herpetology, 33, 567–576.

Bovero, S. 1991. Analisi del comportamento riproduttivo di esemplari sintopici, pedogenetici e metamorfosati, di *Triturus alpestris apuanus*. M.Sc. thesis, University of Turin.

Cei, G. 1943. Ricerche biologiche e sperimentali sul ciclo sessuale annuo dei tritoni alpestri ("*Triturus alpestris*" Laur.) del Trentino e dell' Alto Adige. Studi Trentini di Scienze Naturali, 23, 189–241.

Darwin, C. 1871. The Descent of Man, and Selection in Relation to Sex. London: John Murray; 1981 reprint, Princeton University Press.

Denoël, M. 1998. The modulation of movement as a behavioral adaptation to extreme environments in the newt *Triturus alpestris cyreni*. Journal of Herpetology, 32, 623–625.

Denoël, M. & Joly, P. 2000. Neoteny and progenesis as two heterochronic processes involved in paedomorphosis in *Triturus alpestris* (Amphibia, Caudata). Proceedings of the Royal Society of London, Series B, 287, 1481–1485. doi:10.1098/rspb.2000.1168.

Denoël, M. & Joly, P. In press. Adaptive significance of facultative paedomorphosis in *Triturus alpestris* (Amphibia, Caudata): resource partitioning in an Alpine lake. Freshwater Biology.

Denoël, M., Poncin, P. & Ruwet, J. C. 2001. Alternative mating tactics in the Alpine newt *Triturus a. alpestris*. Journal of Herpetology, 35, 62–67.

Gabor, C. R. & Halliday, T. R. 1997. Sequential mate choice by multiply mating smooth newts: females become more choosy. Behavioral Ecology, 8, 162–166.

Gilbert, D. G. & Starmer, W. T. 1985. Statistics of sexual isolation. Evolution, 39, 1380–1383.

Gould, S. J. 1977. Ontogeny and Phylogeny. Cambridge, Massachusetts: Belknap Press.

Green, A. J. 1991. Large male crests, an honest indicator of condition, are preferred by female smooth newts, *Triturus vulgaris* (Salamandridae) at the spermatophore transfer. Animal Behaviour, 41, 367–369.

Gütleb, B. 1991. Phalangenregeneration und eine neue Methode zur Individualerkennung bei Bergmolche, *Triturus alpestris* (Laurenti, 1768) (Caudata: Salamandridae). Herpetozoa, 4, 117–125.

Halliday, T. 1977. The courtship of European newts: an evolutionary perspective. In: The Reproductive Biology of Amphibians (Ed. by D. H. Taylor & S. I. Guttman), pp. 185–232. New York: Plenum.

Harris, R. N. 1987. Density-dependent paedomorphosis in the salamander *Notophthalmus viridescens dorsalis*. Ecology, 68, 705–712.

Hedlund, L. 1990. Factors affecting differential mating success in male crested newts, *Triturus cristatus*. Journal of Zoology, 220, 33–40.

Hoikkala, A. 1986. The maintenance of sexual isolation between four sympatric species of the *D. virilis* group. Animal Behaviour, 34, 158–161.

Hollocher, H., Ting, C., Pollack, F. & Wu, C. 1997. Incipient speciation by sexual isolation in *Drosophila melanogaster*: variation in mating preference and correlation between sexes. Evolution, 51, 1175–1181.

Kikuyama, S., Toyoda, F., Ohmiya, Y., Tanaka, S., Matsuda, K. & Hayashi, H. 1995. A sex-attractant in the cloacal gland of the newt, *Cynops pyrrhogaster*. Netherlands Journal of Zoology, 45, 160–162.

Kornfield, I., Smith, D. C., Gagnon, P. S. & Taylor, J. N. 1982. The cichlid fish of Cuatro Cienegas, Mexico: direct evidence of conspecificity among distinct trophic morphs. Evolution, 36, 658–664.

Krenz, J. D. & Sever, D. M. 1995. Mating and oviposition in paedomorphic *Ambystoma talpoideum* precedes the arrival of terrestrial males. Herpetologica, 51, 387–393

McCullagh, P. & Nelder, J. 1989. Generalized Linear Models. 2nd edn. London: Chapman & Hall.

Maekawa, K. & Onozato, H. 1986. Reproductive tactics and fertilization success of mature male Miyabe charr, *Salvelinus malma miyabei*. Environmental Biology of Fishes, 15, 119–129.

Michalak, P. & Rafinski, J. 1999. Sexual isolation between two newt species, *Triturus vulgaris* and *T. montandoni* (Amphibia, Urodela, Salamandridae). Biological Journal of the Linnean Society, 67, 343–352.

Michalak, P., Grzesik, J. & Rafinski, J. 1997. Tests for sexual incompatibility between two newt species, *Triturus vulgaris* and *Triturus montandoni*: no-choice mating design. Evolution, 51, 2045–2050.

Roff, D. A. & Fairbairn, D. J. 1991. Wing dimorphism and the evolution of migratory polymorphisms among the insecta. American Zoologist, 31, 243–251.

Ryan, T. J. & Semlitsch, R. D. 1998. Intraspecific heterochrony and life history evolution: decoupling somatic and sexual development in a facultatively paedomorphic salamander. Proceedings of the National Academy of Sciences, U.S.A., 95, 5643–5648.

Seehausen, O., Van Alphen, J. J. M. & Lande, R. 1999. Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. Ecology Letters, 2, 367–378.

Seliskar, A. & Pehani, H. 1935. Limnologische Beiträge zum Problem der Amphibienneotenie. Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie, 7, 263–294.

Semlitsch, R. D. 1987. Paedomorphosis in *Ambystoma talpoideum*. Effects of density, food, and pond drying. Ecology, 68, 994–1002.

Semlitsch, R. D. & Wilbur, H. M. 1989. Artificial selection for paedomorphosis in the salamander *Ambystoma talpoideum*. Evolution, 43, 105–112.

Shaffer, H. B., Austin, C. C. & Huey, R. B. 1991. The consequences of metamorphosis on salamander (*Ambystoma*) locomotor performance. Physiological Zoology, 64, 212–231.

Smith, T. B. 1990. Comparative breeding biology of the two bill morphs of the black-bellied seedcracker. Auk, 107, 153–160.

Smith, T. B. & Skulason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematic, 27, 111–133.

Tompkins, R. 1978. Genic control of axolotl metamorphosis. American Zoologist, 18, 313–319.

Twitty, V. C. 1966. Of Scientists and Salamanders. San Francisco: W. H. Freeman.

Verrell, P. A. 1990. Frequency of interspecific mating in salamanders of the plethodontid genus *Desmognathus*: different experimental designs may yield different results. Journal of Zoology, 221, 441–451.

Verrell, P. A. & Tilley, S. G. 1992. Population differentiation in plethodontid salamanders: divergence of allozymes and sexual compatibility among populations of *Desmognathus imitator* and *D. ochrophaeus* (Caudata: Plethodontidae). Zoological Journal of the Linnean Society, 104, 67–80.

Voss, S. R. 1995. Genetic basis of paedomorphosis in the axolotl, *Ambystoma mexicanum*: a test of the single-gene hypothesis. Journal of Heredity, 86, 441–447.

Voss, S. R. & Shaffer, H. B. 1997. Adaptive evolution via a major gene effect: paedomorphosis in the Mexican axolotl. Proceedings of the National Academy of Sciences, U.S.A., 94, 14185–14189.

Whiteman, H. H. 1994. Evolution of facultative paedomorphosis in salamanders. Quarterly Review of Biology, 69, 205–221.

Whiteman, H. H. 1997. Maintenance of polymorphism promoted by sex-specific fitness payoffs. Evolution, 51, 2039–2044.

Whiteman, H. H., Wissinger, S. A. & Brown, W. S. 1996. Growth and foraging consequences of facultative paedomorphosis in the tiger salamander, *Ambystoma tigrinum nebulosum*. Evolutionary Ecology, 10, 433–446.

Whiteman, H. H., Gutrich, J. J. & Moorman, R. S. 1999. Courtship behavior in a polymorphic population of the tiger salamander, *Ambystoma tigrinum nebulosum*. Journal of Herpetology, 33, 348–351.

Ziuganov, V. V. & Zotin, A. A. 1995. Pelvic girdle polymorphism and reproductive barriers in the ninespine stickleback *Pungitius pungitius* (L.) from northwest Russia. Behaviour, 132, 1095–1105.