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

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**Abstract**

Paedomorphism, 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, paedomorphism 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.

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**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 (Tomkins 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).
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 sympathy (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 36 females; metamorphs: 28 males and 24 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 generations 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 (Gütle 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>
<thead>
<tr>
<th>Phase</th>
<th>Mating frequency</th>
<th>Isolation asymmetry</th>
<th>Propensity asymmetry</th>
<th>Joint sexual isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \pi_{PP} )</td>
<td>( \pi_{PM} )</td>
<td>( \pi_{PM} )</td>
<td>( \pi_{MP} )</td>
</tr>
<tr>
<td>Displaying males</td>
<td>0.500 (24)</td>
<td>0.813 (16)</td>
<td>0.667 (18)</td>
<td>0.640 (25)</td>
</tr>
<tr>
<td>Success of encounters</td>
<td>0.125 (24)</td>
<td>0.250 (16)</td>
<td>0.111 (18)</td>
<td>0.160 (25)</td>
</tr>
<tr>
<td>Success of displaying males</td>
<td>0.211 (19)</td>
<td>0.308 (13)</td>
<td>0.133 (15)</td>
<td>0.235 (17)</td>
</tr>
<tr>
<td>Success of males transferring spermatophores</td>
<td>0.278 (18)</td>
<td>0.333 (12)</td>
<td>0.143 (14)</td>
<td>0.132 (14)</td>
</tr>
</tbody>
</table>

Means are given ± SE. Sample sizes are given in parentheses. For definitions see Methods.

\( P < 0.05 \)
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 (time from the start of the male displays to the first spermatophore deposition); and female positive response time (proportion of time during which 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),

\[
IA = \frac{MP - PM}{PM + MP}.
\]

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),

\[
PA = \frac{MM - PP}{MM + PP}.
\]
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 \( t \) 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](image-url)
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 heterochronic 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-

### Table 2. Coefficients of correlation between morphological characters of males and three measures of sexual encounter success in female metatmorphs 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)

<table>
<thead>
<tr>
<th>Female response</th>
<th>Male character</th>
<th>Female metatmorphs</th>
<th>Female paedomorphs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>r</td>
<td>t(N-2)</td>
</tr>
<tr>
<td>Female positive response time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dots</td>
<td>35</td>
<td>0.129</td>
<td>0.745</td>
</tr>
<tr>
<td>cloaca</td>
<td>35</td>
<td>0.015</td>
<td>0.087</td>
</tr>
<tr>
<td>tail depth</td>
<td>35</td>
<td>-0.179</td>
<td>-1.043</td>
</tr>
<tr>
<td>length</td>
<td>35</td>
<td>-0.092</td>
<td>-0.531</td>
</tr>
<tr>
<td>body mass</td>
<td>35</td>
<td>0.068</td>
<td>0.395</td>
</tr>
<tr>
<td>Female response latency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dots</td>
<td>32</td>
<td>0.077</td>
<td>0.424</td>
</tr>
<tr>
<td>cloaca</td>
<td>32</td>
<td>-0.031</td>
<td>-0.170</td>
</tr>
<tr>
<td>tail depth</td>
<td>32</td>
<td>-0.229</td>
<td>-1.290</td>
</tr>
<tr>
<td>length</td>
<td>32</td>
<td>0.208</td>
<td>1.164</td>
</tr>
<tr>
<td>body mass</td>
<td>32</td>
<td>-0.064</td>
<td>-0.350</td>
</tr>
<tr>
<td>Encounter success</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dots</td>
<td>36</td>
<td>0.225</td>
<td>1.346</td>
</tr>
<tr>
<td>cloaca</td>
<td>36</td>
<td>-0.088</td>
<td>-0.512</td>
</tr>
<tr>
<td>tail depth</td>
<td>36</td>
<td>-0.301</td>
<td>-1.842</td>
</tr>
<tr>
<td>length</td>
<td>36</td>
<td>-0.056</td>
<td>-0.327</td>
</tr>
<tr>
<td>body mass</td>
<td>36</td>
<td>0.003</td>
<td>0.016</td>
</tr>
</tbody>
</table>

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 spermato-

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**Table 2.** Coefficients of correlation between morphological characters of males and three measures of sexual encounter success in female metatmorphs 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)
nels 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 (Smlitsch & 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 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 paedomorph 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 advantageous genotype to fixation. We observed that 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 (Semlitsch et al. 1999).

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 intermediate step to sympatric speciation (Smith & Skulason 1996; Seehausen et al. 1999), but rather in favour of the maintenance of facultative paedomorphosis as a valuable option.

**Acknowledgments**

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**References**


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