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Low proportions of reproducing hemiclonal females increase the stability of a sexual parasite–host system (*Rana esculenta*, *R. lessonae*)

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Summary

1. The number of reproductive events per lifetime affects all levels of ecological complexity, including the structure and dynamics of species communities. Here we investigate a sexual parasite–host system with two water frog species of which one (*Rana esculenta*, E) is a hemiclonal hybrid that depends on one of its parental species (*R. lessonae*, L) for successful reproduction. Previous theoretical models have shown that relative fecundities are important for the species composition and stability of this system, but empirical data from natural mixed LE-populations were lacking.

2. We used three different methods to estimate the proportion of annually reproducing hybrid and parental females: (a) screening for eggs through a tiny incision into the belly skin, (b) measuring plasma hormone titres from blood samples and (c) counts of egg clumps deposited into the pond.

3. After accounting for the respective adult population sizes of the two female types – calculated from mark–recapture analyses – we found that all three estimates revealed a higher proportion of reproducing L- than E-females. Ratios ranged from 1·43 (method c), to 1·90 (a) to 2·38 (b), with an average of 1·90.

4. We also investigated three potential causes for the fecundity difference (age structure, hybrid sterility and differences in reproductive costs) but could not find compelling evidence for any of them. We hypothesize that size-related differences in energy budgets offer the most probable explanation.

5. The consequences of the observed species differences in annual reproductive success were analysed with a theoretical model. The higher proportion of reproducing *R. lessonae* females increases the possibilities for stable coexistence in mixed LE-populations under a variety of combinations of mating patterns, relative fecundity and larval performance. The results, however, also indicate new limits for coexistence and highlight the paramount importance of larval competition for explaining the composition and dynamics of mixed LE-populations.

Key-words: fecundity, hybridogenesis, lifetime reproductive success, reproductive costs, water frogs.

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Introduction

Life-history theory predicts the optimal allocation of reproductive effort (RE) resulting from trade-offs between present reproduction, survival, growth and future reproduction at the level of the individual (Lessells 1991; Roff 1992; Stearns 1992). At higher levels, RE influences the demographic composition of populations and species, such as age structure and sex ratios, the effective population size (*Ne*), which affects genetic diversity and evolutionary potential, and the net reproductive rate (R_0), which determines changes in population densities. On the level of communities, it follows directly from Lotka–Volterra equations that the dynamics of interspecific competition as well as those in predator–prey and parasite–host systems will

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be influenced substantially by the RE of each participant. Natural systems, however, usually defy Lotka–Volterra predictions because the dynamics of one species is less exclusively determined by the dynamics of the other than assumed by the models (Begon, Harper & Townsend 1996). Hence, the relative importance of the number of reproductive events for the stability of the system is difficult to evaluate.

In this study, we investigate the link between reproduction frequency and community dynamics for a system of two unusually tightly coupled species of Palaearctic water frogs in which one (*Rana esculenta*) is a sexual parasite that requires the gametes of a sexual host (*R. lessonae*) for successful reproduction. Theoretical models have shown that relative fecundities are important for the species composition and stability of this system (Hellriegel & Reyer 2000; Som, Anholt & Reyer 2000), but quantitative data on reproduction frequency under natural conditions were lacking.

THE PALAEARCTIC WATER FROG SYSTEM

Rana esculenta (genotype LR) is originally a hybrid between *R. lessonae* (LL) and *R. ridibunda* (RR) (Berger 1977; Günther 1990). It reproduces through hybridogenesis (Schultz 1969), i.e. it eliminates one of its parental genomes prior to meiosis and produces haploid gametes containing only the unrecombined genome of the other parental species. Because hybrid × hybrid matings usually lead to inviable offspring, due probably to an accumulation of deleterious mutations on the unrecombined genome (Vorburger 2001a; Guex, Hotz & Semlitsch 2002), hybrid populations can persist in sympatry only with the parental species whose genome they exclude. Typically, this is *R. lessonae* (LE system; Uzzell & Berger 1975). Fertilization of R gametes produced by *R. esculenta* with L gametes from *R. lessonae* restores somatic hybridity (LR) in the next generation (Graf & Polls Pelaz 1989).

Species composition in such mixed LR/LL populations is highly variable among ponds, ranging from 5 to 95% *R. lessonae* (Berger 1977; Blankenhorn 1977). Within ponds, however, ratios remain fairly stable over time even when population size fluctuates (Holenweg Peter, Reyer & Abt Tietje 2002). This is surprising, because a number of features can be expected to favour an increase of hybrid proportions. These include: (a) one of the four possible mating combinations results in inviable offspring (LR × LR), and of those three combinations producing viable offspring two (LR × LL and LL × LR) result in *R. esculenta* and only one (LL × LL) in *R. lessonae*; (b) fecundity is higher in *R. esculenta* than in *R. lessonae* females (Berger & Uzzell 1980); and (c) hybrid larvae survive better under most ecological conditions (Semlitsch & Reyer 1992; Semlitsch 1993). Factors counteracting this hybrid superiority include overt and cryptic mate choice in females with a preference for *R. lessonae* males (Abt & Reyer 1993; Reyer, Frei & Som 1999; Roesli & Reyer 2000; Engeler & Reyer 2001) as well as reduced fertility (Günther 1990), decreased fertilization success (Reyer, Niederer & Hettyey 2003) and lower sexual activity in *R. esculenta* than in *R. lessonae* males (Blankenhorn 1977).

In this study we investigate another factor potentially reducing the hybrid's primary fitness advantage: less regular breeding. For amphibians, including water frogs, it is well known that not all females reproduce every year (Sjögren 1988; Schmidt, Schaub & Anholt 2002). If the percentage of biennial or less frequent breeding were higher in hybrid than in parental females, this would increase the proportion of *R. lessonae* produced and contribute to maintaining the observed stability in mixed populations. Specifically, we asked the following three questions:

1. Is the proportion of females reproducing smaller in *R. esculenta* than in *R. lessonae*?

2. Does reproduction in one year reduce the probability of reproduction in the next year more in *R. esculenta* than in *R. lessonae*?

3. How do the observed differences in reproduction affect the composition and stability of mixed LE populations?

While the second question investigates potential causes for species differences in the costs of reproduction that might be found under the first question, the third question addresses the consequences of such difference for population dynamics.

Methods

STUDY AREA

The study was performed in 1997 and 2000 near Zurich airport, Switzerland, where several ponds lie within a few hundred metres. Species proportions vary among ponds (Holenweg Peter *et al*. 2002), but mixed populations with about equal numbers of *R. lessonae* (LL) and *R. esculenta* (LR) plus a few *R. ridibunda* (RR) are typical for most ponds, including the two used for this study. *R. ridibunda* probably have been introduced artificially into this area (Grossenbacher 1988) as RR offspring that do arise from LR × LR matings usually die during the larval or juvenile stage, both in the laboratory and the field (Vorburger 2001b; Guex *et al*. 2002). The pond investigated in 1997 consisted of water-filled vehicle ruts with a total surface area of *c*. 900 m^2; the pond investigated in 2000 is a fenced peat bog measuring *c*. 100 m^2. Further details about the study area and characteristics of the ponds are given by Holenweg Peter (2001) and Holenweg Peter *et al*. (2002).

CATCHING, MEASURING AND MARKING ANIMALS

At the fenced pond, frogs were caught in pitfall traps along both sides of the fence (Brown 1997). Buckets were checked daily during the breeding season (14 April–16 June 2000; = spring) and every other day during the post-breeding season (18 June–22 August 2000; = summer). In addition, we spent 7 nights each at the beginning of the breeding season (14 April–1 May) and after breeding (24 July–24 August) catching in the pond and within the fenced area to obtain frogs that had hibernated inside the fence, had already arrived at the pond before the fence was closed and/or had not yet left the pond when the field study was terminated. The same technique was used in 1997 at the unfenced pond, where frogs were caught during three periods: breeding season (11 May–4 June), post-breeding season (28 July–2 August) and autumn (2 October).

All caught animals bigger than 45 mm snout–vent length (SVL) were classified by sex and species. Males were distinguished from females through the presence of vocal sacs and large thumb pads (D'Istria *et al*. 1972). The species were distinguished by the inner metatarsal tubercle (callus internus), which differs in size and form (Berger 1977). In cases of species uncertainty (57 of the 370 caught individuals), 3–5 μL lymph was taken with a glass capillary from an incision made into the web of a hind foot. The lymph was subjected later to enzyme electrophoresis to determine the type of the frogs from albumin patterns (Uzzell & Berger 1975). In females, the foot web incision was then lengthened slightly and the emerging drop of blood was collected in a heparinized microvette (Microvette CB 300, Sarstedt, Germany) for later hormone analysis (see below).

All frogs were weighed with a spring balance (Pesola AG, Baar, Switzerland) to the nearest 0·5 g and snout–vent-length (SVL) was determined to the nearest 0·5 mm with a vernier calliper. Before releasing the animals, they were marked individually by inserting a 0·1 g passive integrated transponder (PIT tag; Trovan ID 100, Euro ID, Weilerwist, Germany) subcutaneously into the lateral lymph sac (Sinsch 1992). This marking technique allows easy identification with a hand reader (Trovan LID 500, Euro ID). Every subsequent recapture was recorded, but to avoid too much stress and injury blood samples were taken only twice per individual, once during the breeding and once during the post-breeding season.

IDENTIFICATION OF REPRODUCTIVE STATUS

To identify the reproductive status we used a combination of one direct (a) and two indirect measures (b, c).

(a) Skin incision

A *c*. 2 mm long incision into the skin of the belly side at the height of the ovary allowed direct visual detection of the eggs. Results from this technique are our reference when we speak of females with or without eggs.

(b) Body condition

From body mass and SVL we calculated the index BCI = $10^4 \times$ weight/SVL^3, which is used widely to express body condition in both invertebrates and vertebrates, including amphibians (Jakob, Marshall & Uetz 1996; Lüddecke 1997; Green 2001).

(c) Testosterone titre

Blood for hormone analysis was taken soon after catching (see above) to ensure that androgen levels did not drop due to the captivity stress (Licht *et al*. 1983; Paolucci *et al*. 1990). Immediately after the blood had been taken, it was centrifuged at 1000 rpm for 10 min in a mobile centrifuge (Microcentrifuge, Denver Instruments, Norfolk, UK), powered by a car battery. The plasma was transferred into an Eppendorf tube and kept on ice until it was stored in a deep freeze at –20 °C. Analysis of the testosterone titre was performed with standard radioimmunoassay (RIA) techniques (Moreno *et al*. 1980; Chard 1990) using AK 8/3 as an antibody. Every sample was tested twice. Depending on the amount of plasma available, the lowest testosterone concentration that could be interpreted with confidence ranged from 1·12 to 5·89 ng/mL.

Details of these methods, including those for the testosterone analysis are described by Reyer & Bättig (2004). The combination of these three methods not only allows measurement of reproductive status within a particular season (question 1), but – when applied in autumn – also to determine whether or not females are likely to reproduce the following year (question 2). This is because reproduction in temperate-zone anurans follows an annual cycle which includes restoring of high sex hormone titres (especially androgens) and completion of vitellogenesis prior to hibernation in those females that will breed the next season (Follet & Redshaw 1974; Licht *et al*. 1983; Pierantoni *et al*. 1984; Gobetti *et al*. 1990; Jørgensen 1992).

MEASURING REPRODUCTIVE OUTPUT

Ponds were searched for eggs every day during the breeding season and every other day during the post-breeding season. Every discovered egg mass was marked by a numbered bamboo stick to avoid counting an egg mass twice. Because no spawning occurred in arenas the vast majority of clutches were well separated, even when they were broken up into several small scattered egg masses. In those cases eggs were assigned to the same clutch if they were in the same developmental stage, found within a radius of 2 m and originated from the same female type. For determining which female type had laid the eggs, we collected a few eggs from every clutch and identified maternity via allozyme electrophoresis for 6-phosphogluconat-dehydrogenase (EC number 1·1·1·44) and for lactate dehydrogenase (EC number 1·1·1·27; Richardson *et al*. 1986). As the hybrid only produces R-gametes, this method cannot distinguish between *R. esculenta* and *R. ridibunda* mothers. For the analyses, we therefore had to pool the two parent types. However, with a species ratio of 9 : 1 (see Results), the error is probably small, and we can assume safely that most clutches with R-eggs must have been laid by *R. esculenta* females.

CONTROL ANIMALS

In 1997, we kept 33 individually marked females (16 *R. lessonae*, 17 *R. esculenta*) in four fenced ponds (each 2·5 × 1·5 m) on the university campus where they could engage in normal mating activity with LL and LR males. These ponds were visited several times per day to check for mating activity. From each female we took several blood samples before and after spawning. This allowed us to relate testosterone titres to the precise date of egg release.

DATA ANALYSIS

Statistics

We used analyses of variance (ANOVA) and covariance (ANCOVA) to test how various measures of reproductive condition and body growth over the season are effected by species (LL, LR), egg-carrying in spring (yes/no), body size (SVL), body condition index (BCI) and time of the year (day or period). Prior to analyses, proportions of females with eggs were transformed angularly by the arcsine of the square-root, and SVL, BCI and testosterone titres were ln-transformed to increase additivity of effects and equality of variance (Snedecor & Cochran 1980). In both 1997 and 2000 several individuals were caught repeatedly during the season, but for all analyses – with the exception of the survival analyses (see below) – we selected only one observation per individual to avoid pseudoreplication. Selection was random, except in cases with incomplete observations. In those cases, complete observations were given priority.

Survival analyses

We used capture–mark–recapture (CMR) methods to calculate weekly survival rate, and thus accounted for the problem that not every frog was captured in every week (Lebreton *et al*. 1992; Nichols 1992). We verified that our data met the assumptions that every marked individual in the population has the same probability of surviving and of being recaptured at any occasion using the goodness-of-fit (GOF) test provided by program RELEASE (Burnham *et al*. 1987). The test showed that the model assuming time-dependent survival and recapture rates was adequate (1997: test 2 + 3: $\chi^2 = 10{\cdot}1$, d.f. = 15, $P = 0{\cdot}81$; 2000: $\chi^2 = 11{\cdot}2$, d.f. = 21, $P = 0{\cdot}96$). We followed basic model selection methodology (Lebreton *et al*. 1992; Burnham & Anderson 1998) and evaluated the evidence for each model using Akaike's information criterion (AICc). The model with the lowest value of AICc provides the best balance between overfitting (hence loss of precision) and underfitting (hence bias, Burnham & Anderson 1998). The Akaike weights give the relative support that a given model has compared to the other models in the set (Burnham & Anderson 1998: 123).

MODELLING COEXISTENCE AND SPECIES COMPOSITION

We adapted a recent model for a single mixed LE population (Hellriegel & Reyer 2000) to additionally include the proportion of *R. lessonae* (p_L) and *R. esculenta* females (p_E) reproducing. The new model was based on the same equations and parameter values as the previous one, but replaced the original parameters c_L and c_E, i.e. the number of L- and E-hatchlings per female and season, by the products $c_L \times p_L$ and $c_E \times p_E$. A simple case for which an equilibrium species ratio can be determined is that of competitive equality of L- and E-tadpoles ($k_E = k_L$; cf. Hellriegel & Reyer 2000). Under these conditions the species ratio only depends on the preferences of L- and E-females for L-males (m_{LL}, m_{EL}) and on the quotient comparing the ratio of reproducing females (r_p: $= p_L/p_E$) with the 'clutch size' ratio (r_c: $= c_E/c_L$):

Proportion of *R. lessonae* =

$$\frac{2m_{EL} - m_{LL}\left[\dfrac{r_p}{r_c}m_{LL} - m_{EL}(3 - m_{LL})\right]}{m_{LL}\left[\dfrac{r_p}{r_c}(1 - m_{LL}) - m_{EL}(2 - m_{LL})\right]} \quad \text{eqn 1}$$

Note the reversed species order in the two ratios.

Results

In total, we caught and marked 370 different females, 267 in 1997 and 103 in 2000. With only 23 individual *R. ridibunda* (6·2% of the total and 10·6% of the *R. esculenta* + *R. ridibunda* population), this species was too rare to be included in the analyses. Among the remaining 347 females, the percentage of the hybrid *R. esculenta* was slightly higher than that of the parental species *R. lessonae* (55·7% in 1997, 55·4% in 2000).

SEASONAL PATTERN OF REPRODUCTIVE CONDITION

In the field, egg clutches were found between early May and late June (bars in Fig. 1b), with no significant temporal difference between *R. lessonae* and *R. esculenta* (Cochran's linear trend value = 0·815, d.f. = 1, $P = 0{\cdot}367$). The testosterone titre decreased from its highest values at the beginning of the breeding season in mid-April to the lowest values in mid-June and early July when breeding is over. Then it rose again during late July and August until reaching a plateau in September/October (Fig. 1a). This seasonal pattern follows a polynomial function of the third order, with the linear, the quadratic and the cubic element all being significant (Table 1a). In addition, there is a highly significant egg

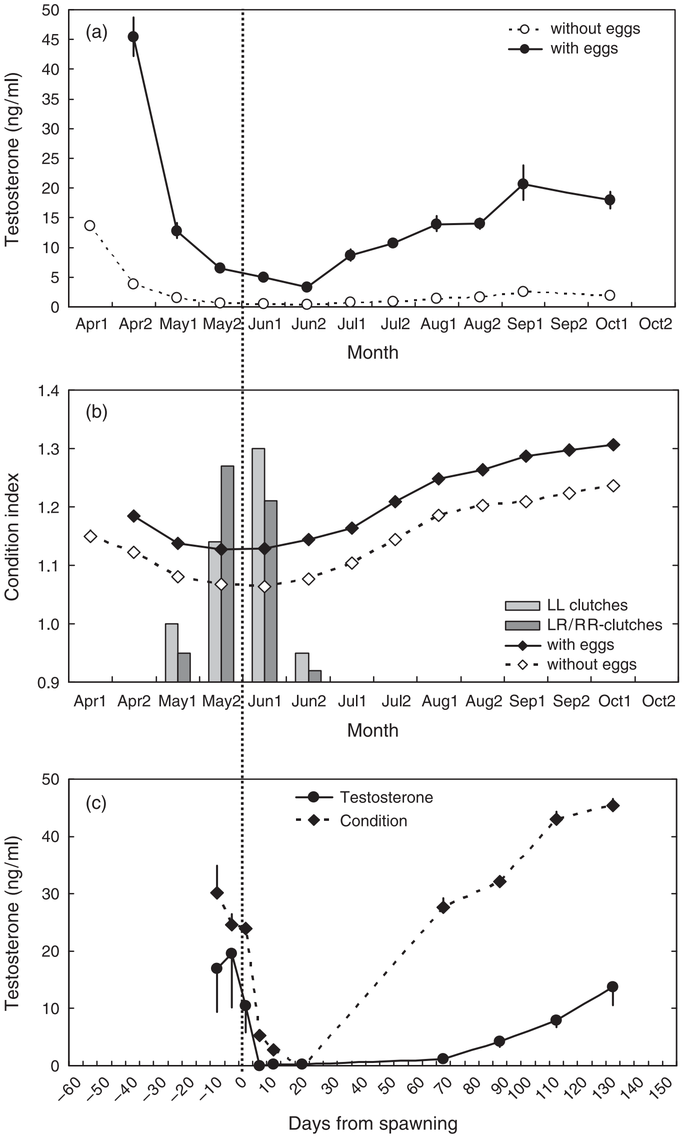

Fig. 1. Testosterone titres (a) and body condition indices (b) of females caught between the first half of April (Apr1) and the second half of October (Oct2). Shown are least square means (± SE) of the estimates from the two ANCOVA in Table 1, plotted against the half month (i.e. days 1–15 or 16·31) during which the respective females were caught; (b) also shows the number of clutches laid by *R. lessonae* (LL) and *R. esculenta* (LR) and/or *R. ridibunda* (RR) between early May and late June. Classification of females as 'with eggs' or 'without eggs' is based on whether or not we saw eggs through the skin incision. Where error bars are not visible they are so small that they are covered by the symbols. In (c) testosterone titres and condition indices of captive females are plotted against the time from the day of spawning (= day 0). The *x*-axes in all three graphs span the same period (1 April–31 October). The dotted line, connecting the day of spawning in captive frogs (c) and the peak of the breeding season in nature (a, b), allows comparison between testosterone titres, respectively, condition indices, in the two situations. Means per half month (a,b), respectively, 10-day intervals (c) are based on the following average sample sizes: (a) 11 for females with eggs, 12 for females without eggs; (b) 12 for females with eggs, 16 for females without eggs; (c) 6.

effect. Overall, females with eggs have much higher testosterone titres than females without eggs, but the extent of this difference varies with the time of the year. It is highest during the breeding season in spring, lowest after breeding in summer and intermediate in autumn (Fig. 1a). There was also a tendency ($P = 0{\cdot}071$) for testosterone titre to be affected by the condition index: females in better body condition show higher hormone concentrations. Condition itself shows a similar temporal pattern as testosterone (Fig. 1b, Table 1b). Again, values are significantly higher in females with than without eggs.

The close link between reproductive activity and testosterone titres and body condition, respectively, is also obvious in the captive control animals, for which the precise spawning day of individually marked females is known (Fig. 1c). Hormone levels and condition indices dropped sharply after egg-laying, remained low for at least the next 3 weeks and reached high values again about 4 months later. Thus, the seasonal pattern was the same as in the field. It should be noted, however, that Fig. 1c displays the time-course for individual females changing from 'with eggs' to 'no eggs' after

Table 1. Results from two ANCOVA testing for the effects of species, eggs in spring and their two-way interactions on testosterone titres (a) and condition index (b). In both analyses, snout–vent-length (SVL) at the beginning of the season and day of the season when the measure was taken were entered as covariables; in the testosterone analysis body condition index was also included. Because of the polynomial relationship between testosterone, respectively, condition, and date (cf. Fig. 1), the day of the season was also entered as a quadratic (day^2) and cubic term (day^3). Day 1 = 1 April

	(a) Testosterone (R^2 = 0·332)				(b) Condition index (R^2 = 0·173)			
Source	Sum-of-squares	d.f.	*F*	*P*	Sum-of-squares	d.f.	*F*	*P*
Species	3·2	1	0·76	0·384	0·0	1	0·06	0·808
Eggs (in spring)	191·2	1	45·43	< 0·001	0·2	1	11·35	0·001
Species × eggs	1·1	1	0·2	0·616	0·0	1	0·30	0·586
SVL	10·6	1	2·51	0·114	0·0	1	0·83	0·362
Condition index	13·8	1	3·29	0·071				
Day	86·5	1	20·56	< 0·001	0·2	1	9·83	0·002
Day^2	66·0	1	15·68	< 0·001	0·2	1	11·74	0·001
Day^3	47·1	1	11·1	0·001	0·2	1	9·57	0·002
Error	1018·5	237			5·1	301		

spawning and back to 'with eggs' in autumn. In contrast, Fig. 1a,b compares the seasonal patterns of two female classes (with or without eggs) comprising different individuals at any one point in time. Testosterone titre and condition index were not affected by body size (SVL), species or species–eggs interaction (Table 1).

PROPORTIONS OF REPRODUCTIVE FEMALES

There was a significant difference between hybrid and parental species in the proportion of females in reproductive condition (Fig. 2, Table 2a). Throughout the year, eggs were detected through the skin incision in a higher proportion of *R. lessonae* than *R. esculenta* females. The difference seemed more pronounced in spring (76·9% vs. 40·5%) than in summer and autumn, but the species–period interaction was not significant. Period alone, however, had a significant effect, with a higher proportion of females carrying eggs in spring and autumn than in summer (*P* = 0·008 and *P* = 0·077; Scheffé's test) and no difference between spring and autumn (*P* = 0·200). The percentage of individuals in which we were uncertain about presence or absence of eggs differed neither with species or period nor with their interaction (Table 2b).

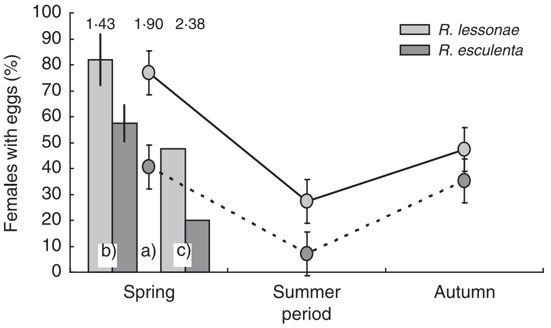

Fig. 2. Mean percentage of *R. lessonae* (light grey) and *R. esculenta* (dark grey) females with eggs in spring, summer and autumn. Dots represent least square means (± SE) from the ANOVA in Table 2a based on egg screening through the skin incision. For spring, these values (a) are compared with means from counting egg clumps (b) and analysing testosterone titres (c). The error bars in (b) indicate the range of values obtained from different estimates. Numbers above (a, b, c) show the ratio of reproductive *R. lessonae* females/ reproductive *R. esculenta* females obtained from the respective method.

Two independent measures confirmed the higher rate of reproduction in parental as opposed to hybrid females. First, when the number of L- and R-egg masses found in the ponds is related to the species-specific female population size in spring, as calculated from the mark–recapture data, we find that, on average, 82·0% (range 72·1–91·8%) of *R. lessonae* and 57·5% (range 51·3–65%) of *R. esculenta* had spawned (bars b

Table 2. Results from two ANOVA testing for the effects of species, three periods of 2 months each (spring: mid-April–mid-June; summer: mid-June–mid-August; autumn: mid-August–mid-October) and their two-way interaction on the percentage of (a) females with eggs and (b) cases in which presence or absence of eggs could not be identified unambiguously. Years (1997, 2000) were used as replicates

	(a) % with eggs (R^2 = 0·862)				(b) % unclear (R^2 = 0·213)			
Source	Sum-of-squares	d.f.	*F*	*P*	Sum-of-squares	d.f.	*F*	*P*
Species	1571·8	1	11·04	0·016	2·5	1	0·01	0·929
Period	3468·8	2	12·38	0·008	114·9	2	0·20	0·823
Species × period	310·7	2	1·09	0·394	344·6	2	0·60	0·577
Error	854·6	6			1709·6	6		

in Fig. 2). The ranges are based on minimum and maximum estimates of clutch numbers in cases where it was difficult to decide whether neighbouring clumps of eggs originated from the same or different females. Secondly, when the number of parental and hybrid females with testosterone titres ≥ 20 ng/mL are related to the respective population sizes in spring, we find that 47·5% of the *R. lessonae* and 20·0% of the *R. esculenta* females were in reproductive condition (bars c in Fig. 2). These figures are definitely too low as 20 ng/mL is a very conservative threshold for carrying eggs (Reyer & Bättig 2004). Nevertheless, the analysis confirms the higher proportion of reproductively active females in *R. lessonae* than in *R. esculenta.* Depending on the method, ratios between the two species range from 1·43 (clutch numbers) to 1·90 (skin incision) to 2·38 (testosterone titres).

Among those individuals that do carry eggs early in the season there was no species-specific difference in reproduction in either the field or the lab (both P = 1·00, Fisher's exact probability test). In both species about 80% (laboratory) to 90% (field) of the initially gravid females did actually spawn. These results are based on 32 individually marked females from the field that could be captured and screened for eggs twice within a few weeks and on 24 gravid females that were closely monitored in the laboratory.

COSTS OF REPRODUCTION

Survival

For both years of the field study, model selection favoured the model accounting for variable recapture success over time and species differences in survival (model 7, Table 3a and model 2, Table 3b). In 2000, the model accounting for different recapture methods was slightly better supported by the data than the model allowing for time dependence only (model 1, Table 3b). The recapture rates ranged from 0·14 (95% confidence interval: 0·04–0·41) to 0·38 (0·22–0·58) in 1997. In 2000, they ranged from 0·10 (0·04–0·23) to 0·43 (0·26–0·59) for weeks in which both capture methods were applied, and was 0·03 (0·02–0·08) for weeks when only the pitfall trapping was conducted. In 2000, the weekly survival rates were 0·83 (0·74–0·90) for *R. lessonae*, and 0·91 (0·82–0·96) for *R. esculenta*. In 2000, the survival rates were 0·94 (0·89–0·97) for *R. lessonae*, and 0·98 (0·94–0·99) for *R. esculenta*.

In the field study, there was no evidence that females carrying eggs differed in survival from females without eggs. The models allowing for such a difference were all poorly supported by the data (models 1–6, Table 3a, and models 4–7, Table 3b). In contrast, the 33 control animals in captivity suggested a tendency for worse survival in females that had reproduced than in those that had not (χ^2 = 2·694, d.f. = 1, P = 0·101), but the sample size is too small to test for potential species differences in this tendency.

Table 3. Summary of model selection for the capture-mark-recapture analysis of weekly survival, ϕ, and recapture probabilities, P, in 1997 and 2000. The subscripts denote the factors included in a particular model. We were interested in potential differences in survival between females carrying eggs and those without eggs. Individuals for which we lack data on the presence of eggs entered the analysis as a third group to increase the sample size and thus allow a more precise estimation of the recapture probabilities. We analysed survival in relation to time (time), egg category (egg), species (sp.) and their interactions, symbolized by *. The recapture probabilities, P were modelled as either variable over time (time) or constant (.). In 2000, we captured frogs both by hand at night and with pitfall traps along a fence. For this data set, we thus considered an additional recapture model that assumed constant recapture rates during weeks in which only traps were used to catch frogs, and variable recapture rate during the remainder of the study period (method/t). We ran all models in program MARK (White & Burnham 1999), and based model selection on Akaike's information criterion (AICc), where a lower value indicates a better model. Delta AICc gives the difference in AICc between the current model and the best model (in bold type); the Akaike weights assess the relative support that a given model has from the data, compared to the other models in the set; K is the number of parameters of a given model. Finally, the deviance is the difference in –2 log-likelihood between the current model and the saturated model, where the saturated model is the one where the number of parameters equals the sample size

Model	AICc	Delta AICc	Akaike weights	K	Deviance
(a) 1997 (N = 161 individuals)					
1 $\phi_{sp*time*egg}P_{time}$	423·30	62·72	0·00	43	108·09
2 $\phi_{sp*time*egg}P_{.}$	432·53	71·95	0·00	43	117·32
3 $\phi_{sp+time+egg}P_{time}$	372·72	12·15	0·00	17	131·27
4 $\phi_{sp*egg}P_{time}$	368·55	7·97	0·02	13	136·54
5 $\phi_{sp+egg}P_{time}$	364·68	4·10	0·11	11	137·25
6 $\phi_{egg}P_{time}$	366·63	6·05	0·04	10	141·45
7 $\phi_{sp}P_{time}$	**360·58**	**0·00**	**0·83**	**9**	**137·62**
(b) 2000 (N = 86 individuals)					
1 $\phi_{sp}P_{time}$	422·57	0·61	0·39	13	220·73
2 $\phi_{sp}P_{method/t}$	**421·96**	**0·00**	**0·53**	**10**	**227·27**
3 $\phi_{sp}P_{.}$	472·17	50·21	0·00	3	292·98
4 $\phi_{sp+egg+time}P_{method/t}$	446·76	24·81	0·00	22	221·31
5 $\phi_{sp*egg}P_{method/t}$	430·60	8·65	0·01	14	226·30
6 $\phi_{sp+egg}P_{method/t}$	426·50	4·55	0·05	12	227·08
7 $\phi_{egg}P_{method/t}$	429·25	7·29	0·01	11	232·21

Growth

There was a marked effect of reproduction on the seasonal increase in snout–vent-length (SVL) (Table 4a). Females with eggs in spring grew 2·7 times less (5·4% vs. 14·6%) during the season than individuals not having eggs in spring (P = 0·029). Growth also depended on body size at the beginning of the season (P = 0·005): the larger the animals, the lower was the percentage of increase in SVL between spring and autumn. However, when size is accounted for growth did not differ between *R. lessonae* and *R. esculenta,* as indicated by the absence of significant species and species × eggs effects. There was also no significant effect of the time span between the first and the second measurement (P = 0·748). This

Table 4. Results from two ANCOVA testing for the effects of species, eggs in spring and their two-way interactions on (a) growth (= % increase in SVL) and (b) reproductive condition in autumn, measured by the factor scores from a principal component analysis performed on testosterone titres and body condition indices of animals caught in the field. Snout–vent-length (SVL) at the beginning of the season and time were entered as covariables. In (a) time refers to the number of days between the first and the second SVL measurement, in (b) it is the day of the season when the sample for testosterone analysis was taken and the condition index was measured

	Growth ($R^2 = 0{\cdot}426$)				(b) Repr. cond. ($R^2 = 0{\cdot}189$)			
Source	Sum-of-squares	d.f.	F	P	Sum-of-squares	d.f.	F	P
Species	0·1	1	0·00	0·964	0·2	1	0·21	0·654
Eggs (in spring)	239·3	1	5·28	0·029	0·8	1	0·87	0·360
Species × eggs	107·0	1	2·40	0·136	0·0	1	0·00	0·974
SVL	418·3	1	9·22	0·005	0·0	1	0·03	0·872
Time	4·8	1	0·11	0·748	5·1	1	5·43	0·028
Error	1270·5	28			26·8	28		

reflects only the small variability ($CV = 8{\cdot}7\%$) in days between the two measurements.

Future reproduction

As well as a probable effect on fecundity resulting from these differences in somatic growth, we detected no direct influence of present on future reproduction in either direct or indirect measures of reproductive condition. A direct measure came from 35 females for which presence and absence of eggs could be determined unambiguously in both spring and August. Of those with eggs in spring ($N = 21$), 42·9% had eggs in late summer again; for those without eggs in spring ($N = 14$) the corresponding figure was 35·7%. These proportions are not significantly different ($\chi^2 = 0{\cdot}179$, d.f. = 1, $P = 0{\cdot}673$).

As an indirect measure of reproductive condition in autumn, we used hormone titres and condition indices. As these two variables are correlated and, hence, represent no independent measures of the presence or absence of eggs (cf. Table 1) we replaced them by the factor scores obtained from a principal component analysis (PCA). These scores neither differed between *R. lessonae* and *R. esculenta* nor with presence or absence of eggs in spring or the species–egg interaction (Table 4b). There was also no influence of body size. The only significant effect came from the date: the later in the year the measurements were taken, the higher were the reproductive condition scores. This only confirms the temporal patterns of testosterone and body condition illustrated in Fig. 1.

COEXISTENCE AND SPECIES COMPOSITION

When the proportions of reproducing females found in this study were entered into the modified model of Hellriegel & Reyer (2000), the following predictions for coexistence were obtained. Without competitive differences between hybrid and parental larvae ($k_E = k_L$, Fig. 3a,b), the equilibrium species ratio depends only on the combination of female preferences for L-males (m_{LL}, m_{EL}) and the relative reproductive output of the two species (r_p/r_c, see eqn 1 in Methods section). When the two species reproduce equally, i.e. $r_p/r_c = 1$ or $p_l \times c_L = p_E \times c_E$, coexistence is possible as long as the preference of L-females for L-males is below 95% (Fig. 3a). This latter restriction is not necessary if the hybrid is reproductively superior (cf. Fig. 3b with $c_E/c_L = 2$ in Fig. 3 of Hellriegel & Reyer 2000). If *R. lessonae* is reproductively superior the possibilities for coexistence are reduced for $r_p = 1{\cdot}5 \times r_c$ (Fig. 3b) and coexistence is impossible as soon as $r_p \geq 2{\cdot}8 \times r_c$ (inset Fig. 3b). The hybrid remains in the system only if L-female preference for L-males is random or moderate ($0{\cdot}5 < m_{LL} < 0{\cdot}8$) and E-female preferences are moderate or high ($m_{EL} > 0{\cdot}5$) (Fig. 3b). Competitive superiority of the hybrid clearly favours mixed populations and those dominated by the hybrid (Fig. 3c,d). If the competitive superiority of *R. esculenta* tadpoles ($k_E = 1{\cdot}5k_L$) is counteracted by the reproductive superiority of *R. lessonae* ($r_p = 1{\cdot}5 * r_c$) the range of stable coexistence is increased, but also the occurrence of pure *R. lessonae* populations when compared to the cases of reproductive equality ($r_p = r_c$) (cf. Fig. 3c,d) and of reproductive hybrid superiority (cf. Fig. 3d with $c_E/c_L = 2$ in Fig. 3 of Hellriegel & Reyer 2000).

Discussion

SEASONAL PATTERN OF REPRODUCTIVE CONDITION

Our results agree with those from previous studies in showing that in temperate zone anurans reproductive condition follows a seasonal pattern (Gobetti *et al*. 1990; Jørgensen 1992). High testosterone titres in spring are followed by a marked drop around the time of spawning and a subsequent recovery period in late summer and autumn (Fig. 1a). At all times, hormone values are significantly higher in females with than in those without eggs (Table 1a). Data from our captive control animals show that testosterone titres decrease steeply prior to and around spawning. This offers an explanation

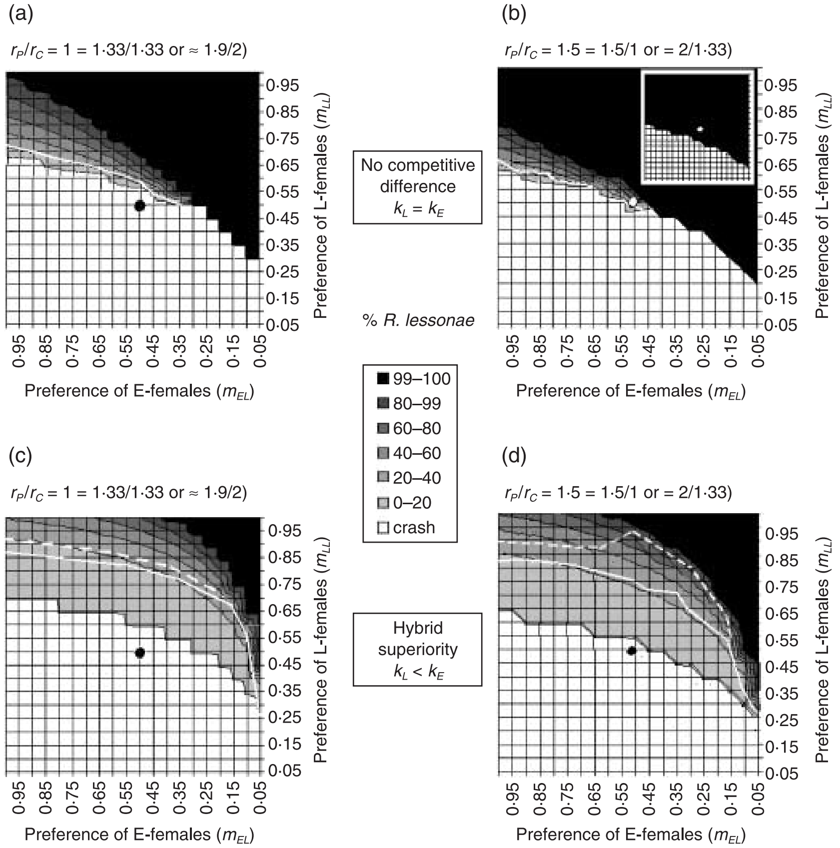

Fig. 3. Percentage of *R. lessonae* (L) in relation to (i) fecundity ratio ($r_c = c_E/c_L$ = E clutch size/L clutch size); (ii) ratio of females reproducing ($r_p = p_L/p_E$ = proportion L females/proportion E females); and (iii) larval competitive abilities (k_L, k_E) for different combinations of female preferences for L-males (m_{LL}, m_{EL}). A horizontal comparison between graphs demonstrates the effect of an increased proportion of reproducing L-females under symmetric (a, b) and asymmetric (c, d) larval competition. A vertical comparison shows the influence of increased competitive ability of hybrid larvae with equal (a, c) and unequal (b, d) proportions of reproducing females. Parameter values: starting point was a mixed population of 50 *R. lessonae* and 50 *R. esculenta* individuals. Clutch size ratios r_c were chosen from the observed range between 1·33 (Abt 2003) and 2 (Berger & Uzzell 1980). Ratios of reproducing female proportions were chosen in accordance with the data reported in this study: we assumed p_L = 0·8, p_E = 0·6 for r_p = 1·33, p_L = 0·84, p_E = 0·56 for r_p = 1·5 and p_L = 0·8, p_E = 0·4 for r_p = 2. Competitive equality implied $k_L = k_E$ = 5000, hybrid superiority $k_E = 1{\cdot}5 * k_L$ = 7500. The inset in (b) resulted from r_p/r_c = 2·8 (p_L = 0·84, p_E = 0·3). White lines: below solid line population sizes and species ratio are stable, below broken line the ratio varies by up to 10% (percentages then are means over the last 10 of 1000 years). In (a) (b) the species ratio is always stable. Black/white dot: indicates the point of no selective mate choice to facilitate comparisons between graphs.

of why reported relationships between testosterone titres and presence of eggs vary greatly, ranging from 4·5 ng/mL (Carnevali *et al.* 1993) to 74·3 ng/mL (Licht *et al.* 1983). These differences probably reflect differences in the time to spawning. Unless this time is known precisely and included in the analyses (Table 1a), hormone titres are unlikely to be good indicators of the presence or absence of eggs.

Body condition indices also were higher in females with eggs than in those without, and they followed a similar temporal pattern (Fig. 1b, Table 1b), but differences were less pronounced than in testosterone titres. This probably has both methodological and biological reasons. In terms of methods, reliability of body condition indices calculated from mass/length relationships depends on a large number of assumptions which in many studies are not met (Jakob *et al.* 1996; Green 2001). In terms of biology, evaporative water loss in water frogs can reduce body weight by as much as 20% (Sinsch 1983; G. Abt, unpublished data). This is of the same magnitude as the weight gain resulting from ovaries with ripe eggs (Redshaw 1972). Hence, measures of mass and body condition will be affected strongly by the time the frogs have spent on land or in water prior to catching.

SPECIES DIFFERENCES IN REPRODUCTIVE STATUS? (QUESTION 1)

Testosterone titres, condition indices and the time of spawning did not differ between the species, nor were hormones and body condition affected by body size (SVL), species or species–eggs interaction (Fig. 1b, Table 1). This suggests that the physiological processes underlying reproduction do not differ between the smaller parental species and the larger hybrid. However, the proportions of reproducing females were different (Table 2a, Fig. 2). Our estimates for the ratio of reproductive *R. lessonae*/reproductive *R. esculenta* females ranged from 1·43 (clutch counts) to 1·90 (skin incision) to 2·38 (testosterone), with an average of 1·90. There are various potential reasons for this variation. First, screening for eggs through the skin incision tends to overlook existing eggs more often in *R. esculenta* than in *R. lessonae* (23% vs. 11%) which is due probably to the slightly thicker and less transparent skin of the hybrid (Reyer & Bättig 2004). Correcting for these differences decreases the ratio obtained through this method from 1·90 to 1·65. Secondly, the low ratio resulting from the count of egg clumps may be due partly to an overestimate of the hybrid's reproductive output, resulting from the fact that our allozyme electrophoresis could not distinguish between R-eggs in clutches laid by *R. esculenta* and those laid by *R. ridibunda* (see Methods). If we assume that the two female types reproduce in the ratio of their frequency in the population (9 : 1) and reduce the number of *R. esculenta* clutches accordingly, then the ratio of *R. lessonae*/*R. esculenta* reproduction rises from 1·43 to 1·60. Thirdly, the probability of finding egg masses depends on where they are placed. Some of the clutches were laid deep into the pond and were invisible from the surface; others were hidden in the vegetation. If there were species differences in spawning locations this would affect the ratio, but currently we have no data to test this hypothesis. Finally, there may be species differences in the testosterone thresholds necessary for reproduction and in the rate at which existing eggs are resorbed, rather than spawned. However, this theoretical explanation for variation among the results from different methods finds no support from our data. There was no species effect on testosterone titres (Table 1a) and in both species 80–90% of all females with eggs did actually spawn.

REASONS FOR SPECIES DIFFERENCES (QUESTION 2)

Potential reasons for the lower proportion of reproductively active hybrid than parental females include body size, hybrid sterility and costs of reproduction.

Body size

According to Berger & Uzzell (1980), *R. lessonae* reaches sexual maturity at a smaller size (SVL: 42 mm) than *R. esculenta* (SVL: 54 mm). Hence, theoretically, more hybrid than parental females may have been below the critical size. However, the means and lower 95% confidence limits were well above the thresholds of sexual maturity in both *R. lessonae* [52·7 mm (95% limits: 51·6–53·7)] and *R. esculenta* [60·4 mm (58·9–62·0)].

Hybrid sterility

Previous information about fecundity differences between *R. esculenta* and *R. lessonae* yields no clear picture. For male hybrids, observations range from total sterility in dissected individuals (Günther 1990) to reduced fertilization success of hybrid sperm (Reyer *et al*. 2003) to no obvious differences in artificial fertilization experiments (Berger, Rybacki & Hotz 1994). For females, no comparable information is available.

Costs of reproduction

Gonads with ripe eggs weigh up to 20% of the total body mass (Redshaw 1972) and, hence, reproductive costs are to be expected. However, of the three parameters that we used for measuring these costs (survival, growth, future reproduction) only growth was significantly affected (Table 4a). Females with eggs in spring increased their SVL during the season 2·7 times less than females without eggs. Similar, or even more drastic, effects have been reported for other anuran species (e.g. Jørgensen 1986). Because fecundity correlates with body size, reduced growth will affect clutch size negatively in the next season. Other than that, there was no direct effect on future reproduction (Table 4b) and survival (Table 3). An explanation for this surprising result may come from the fact that in the field females carrying eggs do not necessarily spawn. Some proportion may skip reproduction for one season, resorb the eggs and use them as an additional energy resource. This may lead to condition and survival values similar to those of females arriving without eggs, which incurred no costs through egg-laying in spring but also did not benefit from egg resorption later in the season. In the captive control animals, where egg-laying was identified unambiguously, there was indeed a tendency for reproducing females to survive less well than non-reproducing ones.

Neither in the artificial nor in the natural ponds did we find evidence that reproductive costs differ between the two species. None of the species–egg interactions in Tables 1 and 4 were significant. Thus, from this study we have no reason to assume that the lower proportion of reproductive females in *R. esculenta* than in *R. lessonae* (question 1) may result from higher reproductive costs in the hemiclonal hybrid (question 2). However, such costs can vary with changes in food supply and/or body condition and the response to such changes may be species-specific (Clutton-Brock, Guiness & Albon 1983; Bonnet *et al*. 2002). Results from an experiment indeed suggest that the observed species differences in the frequency of annual reproduction arise from differences in energy budgets. Because of its on average lower body masses, *R. lessonae* has a *c*. 50% lower metabolic rate than *R. esculenta* and, hence, can invest more into reproduction (Wälti & Reyer, unpublished).

CONSEQUENCES FOR THE STRUCTURE AND DYNAMICS OF POPULATIONS (QUESTION 3)

Whatever the source and the extent of the reproductive differences between the two species, it does not change the conclusion emerging consistently from all three methods of identifying reproductive status: the proportion of reproducing females is higher (most probably 60–90%) in the parental species *R. lessonae* than in the hybrid *R. esculenta.* Together with female preferences for *R. lessonae* males (Abt & Reyer 1993; Reyer, Frei & Som 1999; Roesli & Reyer 2000; Engeler & Reyer 2001), this difference will reduce markedly the numerical dominance of hybrids expected from other features of the system (see Introduction). When in the model (modified from Hellriegel & Reyer 2000) the relative reproductive success of parental and hybrid females was changed by magnitudes found in this study, stability of mixed LE populations was increased under various combinations of mating patterns, relative fecundity and larval performance. However, our results also indicate new limits for coexistence. With a relatively small fecundity advantage for the hybrid ($r_c = 1{\cdot}33$; Abt 2003) and a higher proportion of parental females reproducing in a given year ($r_p = 1{\cdot}4$–$1{\cdot}9$; this study), *R. lessonae* can reach reproductive superiority, thereby increasing the necessity of competitive superiority for the hybrid (Fig. 3). A further increase in r_p (e.g. to 2·38, the value suggested by hormone titres), together with a further decrease in r_c (e.g. due to lower fertilization success of *R. esculenta* males; Reyer *et al*. 2003) can drive reproductive superiority of the parental species to levels ($r_p/r_c \geq 2{\cdot}8$) where under equal larval competitive abilities mixed populations are no longer possible (inset in Fig. 3b). For these calculations we assumed similar survival rates between species, because empirical studies show conflicting results (lower survival of *R. lessonae*: this study, Anholt *et al*. 2003; higher survival of *R. lessonae*: Holenweg Peter 2001).

According to Fig. 3, a 50% increase in hybrid larval performance (3c,d vs. 3a,b) affects the composition and stability of the population more than a 50% increase in parental reproductive output (3a,c vs. 3b,d). Hence, any explanation for the markedly different species compositions among ponds (see Introduction) requires, above all, information about the outcome of larval competition in relation to ecological conditions. Although this has been measured in several experiments (e.g. Semlitsch & Reyer 1992; Semlitsch 1993), the competitive outcome is totally unknown for natural ponds. From the model predictions of the present study we expect that under certain parameter combinations, total displacement of the hybrids through the parental species is as possible as the original hypothesis that hybrids should increase at the expense of parentals, because of their higher reproductive output and/or better larval survival (see Introduction).

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