Chorus participation by male bullfrogs, *Rana catesbeiana*: a test of the energetic constraint hypothesis

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(Received 13 July 2000; initial acceptance 13 October 2000; final acceptance 28 March 2001; MS. number: A8827)

Chorus tenure is the number of nights that a male anuran spends attending choruses, and dominant tenure is the number of nights that a male adopts a dominant (i.e. territorial) mating tactic. While male bullfrogs that have longer tenure (chorus tenure and dominant tenure) acquire more mates, tenure is believed to be energetically costly. During the summer of 1998, we tested the hypothesis that tenure of bullfrogs is energy constrained, by conducting a feeding experiment to manipulate energetic condition, and by measuring each male's body condition on every night of his chorus tenure. The energetic constraint hypothesis did not adequately predict variation in male chorus tenure. However, male bullfrogs with longer dominant tenures, other things being equal, were in better initial condition, poorer final condition and lost condition more slowly than males with shorter dominant tenures. Feeding had no significant effect on either chorus tenure or dominant tenure. We found evidence that direct selection through endurance rivalry favoured traits in male bullfrogs that increased tenure.

Breeding site attendance is perhaps one of the most important and widespread determinants of male mating success. Positive relationships between mating success and attendance have been found in anurans (reviewed in: Murphy 1992; Halliday & Tejedo 1995), mammals (Campagnà & Le Boeuf 1988; Apollonio et al. 1989), birds (Gibson & Bradbury 1985; Höglund & Robertson 1990) and insects (Banks & Thompson 1985; Snedden 1996). Therefore, a major goal of sexual selection research is to determine which male traits constrain breeding site attendance and how those traits are selected.

Typically, males have a low probability of acquiring a mate on any given night if female arrival at the breeding site is spread out over time and the operational sex ratio is highly male biased (Emlen & Oring 1977). However, males can increase their probability of mating simply by increasing their attendance at breeding aggregations (Gerhardt et al. 1987; Tejedo 1992; Halliday & Tejedo 1995). Thus, traits that increase male persistence or stamina at the breeding site will be favoured by sexual selection through a mechanism called endurance rivalry (Andersson 1994; Murphy 1998).

Although many studies of anurans have found a positive relationship between male attendance at breeding aggregations and mating success, most male anurans display highly abbreviated attendance relative to the entire breeding season (Murphy 1992). Few studies have examined the male traits that could influence male attendance (Green 1990; Murphy 1994b; Lucas et al. 1996; Marler & Ryan 1996; McCauley et al. 2000), and no study has identified endurance rivalry as an important selective force acting upon those traits (Andersson & Iwasa 1996).

Chorus tenure is the length of time, measured in number of nights, that a male anuran attends chorus activity (Murphy 1994a). Males are thought to restrict their chorus tenure because it is costly in terms of (1) increased predation risk (e.g. Ryan et al. 1981), (2) lost foraging opportunities (e.g. Woolbright & Stewart 1987) and (3) increased energy consumption (e.g. Prestwich et al. 1989). Calling is thought to be the most energetically expensive activity undertaken by anurans (Pough et al. 1992). However, in addition to calling, males that participate in chorus activity also expend energy in aggressive interactions with conspecifics, and in inter- and intrachorus locomotion, particularly when the frogs occupy larger bodies of suitable habitat. The energetic cost of chorus tenure, measured as male weight loss over the chorusing season, has been documented for a number of frog species (reviewed in Halliday & Tejedo 1995). We tested the hypothesis that chorus tenure of male bullfrogs is energy constrained.
Murphy (1994b) developed an energetic limitation hypothesis to link the energetic cost of chorus tenure to variation in chorus tenure for male barking treefrogs, *Hyla gratiosa*. He proposed that chorus tenure might be energy constrained: before chorus tenure begins and/or during chorus tenure. However, this distinction is confusing because the effects of male energetic status on chorus tenure cannot be neatly categorized into either of these two time periods. We constructed an energetic constraint hypothesis, analogous to Murphy’s (1994b) energetic limitations hypothesis without the distinction between constraint acting before or during chorus tenure. The hypothesis predicts that, other things being equal: (1) males in better initial energetic condition will have longer chorus tenures than males in poorer initial energetic condition; (2) males that allow their energetic condition to decline more (i.e. increase their condition loss, because of lower expectation of overwinter survival, or because they are better able to recoup the lost body condition after the breeding season) will have longer chorus tenures than males that allow their energetic condition to decline less; (3) males that lose energetic condition more slowly will have longer chorus tenures than males that lose energetic condition more quickly; and (4) males that are more successful foragers will have longer chorus tenures than males that are less successful.

Male bullfrogs display dominant, satellite and opportunistic mating tactics (Howard 1978a). We subdivided chorus tenure into nights spent as a dominant male (dominant tenure) and nights spent as a satellite or opportunistic male (subordinate tenure). The energetic costs of chorus tenure may be more severe for dominant males than for subordinate males (Emlen 1976). If so, predictions 1–4 of our energetic constraint hypothesis should hold true for both chorus tenure and dominant tenure, but not necessarily subordinate tenure. Although chorus tenure and dominant tenure are highly correlated, we conducted parallel analyses for both chorus tenure and dominant tenure. Analysing chorus tenure allows us to make comparisons with other studies, and analysing dominant tenure allows us to examine a variable that may be more relevant to sexual selection than is chorus tenure.

**METHODS**

**Study Location and Species**

We conducted our research within the Wildlife Research Area of Algonquin Park, on Lake Sasajewun (45°35’N, 78°30’W) and the adjacent bodies of water, during the summers of 1998 and 1999 (Fig. 1). Lake Sasajewun (approximate surface area 43.5 ha, maximum depth 9 m) was created during the early 1900s as an artificial impoundment (Shirose 1990). Bullfrogs are large-bodied, prolonged-breeding anurans (Wells 1977) widely distributed across most of the eastern United States and into southeastern Canada. In Algonquin Provincial Park, Ontario, they are near the northern limit of their distribution (Conant & Collins 1998), and mature later and at a smaller size than bullfrogs from more southern populations (Shirose et al. 1993). Bullfrog chourusing on Lake Sasajewun lasts approximately 6 weeks, from early June to late July. Males’ chorus sites are on the margins of the lake (Fig. 1). These sites have been used consistently for the past 5 years (MacDonald 1997; personal observation). Male bullfrogs display dominant, satellite and opportunistic mating tactics (Howard 1978a). However, the large size of Lake Sasajewun made it impossible to devote the time needed to consistently identify the opportunistic and satellite mating tactics; therefore, we classified males in our study only as being either dominant or subordinate (i.e. satellite, opportunistic or in an indeterminate posture). Dominant male bullfrogs vocalize often and defend territories from other male bullfrogs. They maintain an inflated posture with their throats and backs above the surface of the water, and they move less and fight more than subordinate male bullfrogs (Emlen 1976; Howard 1978a). Males that display the dominant mating tactic achieve higher mating success than males that do not (Howard 1978a; MacDonald 1997). We subdivided chorus tenure (the number of nights that a male bullfrog attends the chorus) into nights spent as a dominant male (dominant tenure) and nights spent as a subordinate male (subordinate tenure).

**Data Collection**

We brought all new chorusing male bullfrogs back to the laboratory after nightly chorus censuses had been completed (see below). We measured the snout–urostyle length of each new male with vernier calipers to the nearest 0.1 mm. We repeated this measurement five times to assess its precision, and used the mean in all subsequent analyses. We placed each male in a plastic container and weighed them to the nearest 0.1 g using an electronic scale (Acculab® V-333 or V-400). All males were implanted with a Passive Integrated Transponder ( PIT) tag (see Brown 1997 for a description of the implantation technique) for individual identification between years, and given a unique alphanumeric tattoo on the belly (see Shirose 1990 for a description of the tattooing procedure) for identification within the chourusing season. We did not use anaesthetic during tattooing, because the relaxation of belly muscles brought on by application of a local anaesthetic made tattoo application more difficult and time consuming, and consequently more stressful for the frogs (personal observation). We never observed local infections at the tattoo site of any individual male, and to the best of our knowledge no male died as a result of tattooing. Recovery time prior to release varied according to the feeding experiment treatment group (see below). Fed and sham-fed males were released approximately 8–12 h following tattoo application and released males were returned to their choruses within 1–3 h following tattoo application.

We censused the Lake Sasajewun population of chorusing male bullfrogs each night during the 1998 and 1999 chourusing seasons. We visited every site at which bullfrogs were heard chourusing, as well as those from which bullfrogs chorused during any one of the previous 5...
nights. Bullfrogs were captured by dipnet, either directly from a canoe or from the shoreline, and placed in buckets according to their observed mating tactic (dominant or subordinate). While still at the chorus site, we identified individuals by their unique alphanumeric tattoos, classified them according to observed mating tactic and then weighed them on an electronic scale to the nearest 0.1 g. Variation in weight due to urinary bladder content was assumed to be negligible because bullfrogs are highly aquatic and do not need to use their bladders to store water as is the case in other, more terrestrial anurans (Shoemaker et al. 1992). We then returned known individuals to their chorus site (within approximately 15 min of capture) and placed new chorusers in labeled containers. We identified males that were in amplexus when their chorus was censused either by waiting until the female finished laying her egg mass and netting the male, or if the amplexed pair had not yet started to lay an egg mass, netting them both and gently lifting the male away from the back of the female, and reading his ventral tattoo. We recorded these observations and measurements verbally on a microcassette tape recorder and transcribed them the following day. We varied the order in which we censused choruses from night to night to reduce the chance of missing any males that may have consistently started their nightly chorus participation after we arrived at the chorus site, or vice versa. We censused other bodies of water surrounding Lake Sasajewun (Fig. 1) occasionally during the summer of 1998 to assess the amount of movement between lakes by breeding males. We collected data on tenure, body size, initial body condition and mating success for male bullfrogs that participated in the 1999 chorusing season.

Tests of the Energetic Constraint Hypothesis

A precondition of the energetic constraint hypothesis is that tenure is energetically costly for male bullfrogs. We established the cost of tenure by comparing the mean change in condition (body mass controlling for size) during both chorus tenure and dominant tenure to zero (no change) using one-tailed t tests. We also regressed

Figure 1. Lake Sasajewun, Algonquin Provincial Park, Ontario, Canada, and nearby bodies of water. Numbers represent main bullfrog chorusing sites.
condition change on both chorus tenure and dominant tenure, and we compared the average per-day rate of condition change during chorus tenure and dominant tenure to zero using one-tailed t tests. Since bullfrog chorus attendance is not continuous (MacDonald 1997), we compared males’ average condition change during absences from both chorus tenure and dominant tenure to zero. If tenure is energetically costly and males are leaving to try to recoup lost condition, then males should gain weight during absences (Bevier 1997).

If the energetic constraint hypothesis is true, then males with longer tenures (chorus tenure and dominant tenure) will, all things being equal, display at least one of the following: (1) higher initial energetic condition, (2) lower final energetic condition, (3) more positive rate of condition change, or (4) greater foraging success, relative to males with shorter tenures.

We tested predictions 1–3 by observational means by calculating three measures of condition for each individual bullfrog, and then examining their unique effects on both chorus tenure and dominant tenure through multiple regression analysis. We generated a residual index of body condition (Jakob et al. 1996) by regressing mass versus snout–urostyle length, both measured on each male’s first night of chorus tenure. The resulting regression line gave us a predicted mass for each male (i.e. the solution of the regression equation given the male’s length). On any given night, a male’s condition was the difference between his measured mass and his predicted mass. Therefore, measured masses lying above the regression line give positive values for condition, and measured masses lying below give negative values. Initial condition and final condition were a male’s condition on his first and last nights of chorus tenure, respectively (or on his first and last nights of dominant tenure). We calculated the rate of condition change by subtracting initial condition from final condition and dividing the result by the number of nights spanned by those two measurements (span of tenure). Because the dates on which males started and ended their chorus tenures often differed from the dates on which they started and ended their dominant tenures, their energetic condition on those nights also differed. Therefore, we calculated the three condition variables for both chorus tenure and dominant tenure. We conducted two multiple regression analyses to test for the effects of energetic condition on tenure, one with chorus tenure as the dependent variable and another with dominant tenure as the dependent variable. We entered initial condition, final condition and rate of condition change calculated for chorus tenure as the independent variables in the chorus tenure multiple regression, and we entered the condition variables calculated for dominant tenure in the dominant tenure multiple regression. We excluded from the chorus tenure multiple regression individuals that had only one night of chorus tenure because it was impossible to calculate their rate of condition change. For the same reason, we excluded from the dominant tenure multiple regression individuals that had only one night of dominant tenure.

We tested prediction 4, the effect of foraging success on tenure, by conducting a feeding experiment. After being weighed and marked, each new male (see above) was randomly assigned to one of three treatment groups: fed, sham-fed and released. The released treatment group was a control for the effects of overnight captivity, and frogs in this group were returned to their choruses immediately following marking. Fed and sham-fed frogs were housed communally overnight until the feeding experiment was conducted the day following their initial capture.

We fed each bullfrog in the fed group a meal of two raw chicken hearts (39.040 kJ/heart), each implanted with a freeze-dried cricket (8.154 kJ/cricket; 94.388 kJ/meal). We opened the frog’s mouth with a spatula, placed the chicken heart inside and then allowed him to close his mouth. We then returned the frog to an individual bucket where he swallowed the food. We then repeated the feeding procedure with a second chicken heart.

The sham-fed treatment group was a control for the effects of handling during feeding. We handled sham-fed males in the same way as bullfrogs in the fed treatment group, except that forceps were placed inside the frog’s mouth without food. We examined the effect of handling during the feeding experiment by comparing the released and sham-fed treatment groups with respect to: (1) the number of nights it took males to return to chorusing activity following processing, and (2) whether they returned to the same chorus following processing. We predicted that if the extra handling of the sham-fed treatment group had a negative effect on the frogs’ behaviour, then released males would resume chorusing faster, and more often at the same chorus than sham-fed males.

Our analysis of the effect of the feeding experiment on tenure was conditional upon whether the extra handling received by the frogs involved in the feeding experiment (fed and sham-fed) had an effect on their behaviour. If there was a negative effect of handling during feeding, then we would test the effect of feeding on tenure by comparing mean chorus tenure and mean dominant tenure between the fed and sham-fed treatment groups using t tests. However, if there was no negative effect, then sham-fed and released treatment groups would be pooled as unfed frogs and a categorical variable, feeding status (fed or unfed), would be added to the multiple regression analyses used to test predictions 1–3. We also compared the final condition and rate of condition change of the three treatment groups in order to assess how male bullfrogs allocate energy.

**Evidence of Selection**

If longer tenure is correlated with increased mating success (number of observed matings), endurance rivalry is predicted to favour traits that enable males to extend their tenures (Andersson 1994). If the energetic constraint hypothesis explains a significant proportion of the variance in bullfrog tenure, then there may be evidence for directional selection on its four predictors.

To test whether the benefit of longer tenure observed in previous years (MacDonald 1997) was still evident in 1998, we compared both mean chorus tenure and mean
dominant tenure among three groups of males: (1) males that were observed mating more than once; (2) males that were observed mating only once; and (3) males that were never observed mating.

To test for evidence of directional selection, we calculated selection differentials (representing the changes in character means produced by direct and indirect selection) and selection gradients (representing direct selection alone). We calculated directional selection differentials as the covariances between relative fitness and each of the four predictors, and directional selection gradients as the partial regression coefficients of relative fitness on the four predictors (Lande & Arnold 1983). We calculated relative fitness of each male bullfrog by dividing each measure of absolute fitness (number of observed matings for each male) by the mean absolute fitness (Lande & Arnold 1983). Because final condition might have an effect on mating success the following year, we compared final condition between males that survived to 1999 and were observed mating, and males that survived to 1999 and were not observed mating. We completed all of the above analyses for the four predictors calculated for chorus tenure as well as those calculated for dominant tenure. This allowed us to identify selection acting during chorus tenure or dominant tenure.

Figure 2. Summary of the 1998 bullfrog chorusing season, Lake Sasajewun, Algonquin Park. (a) Males: ■ dominant; □ subordinate; ■+□ total number of unmated males present on that night. (b) Females: ■ mated; □ unmated; ■+□ total number of females present on that night (X±SD nightly number of males=27.9±9.9 males; median nightly number of males=28.5 males; X±SD OSR=9.3±6.2 males:1 female; median OSR=6.6 males:1 female).

Figure 3. Frequency distribution of bullfrog (a) chorus tenure (X±SD number of nights attending choruses=15.75±11.83 nights; median=13 nights, N=79), and (b) dominant tenure (X±SD number of nights adopting a dominant mating tactic=9.00±8.45 nights; median=7 nights, N=79).

Figure 4. Frequency distribution of the number of nights male bullfrogs (a) attended choruses (chorus tenure) and (b) adopted a dominant mating tactic (dominant tenure), expressed as a percentage of the number of nights spanned by each tenure (percentage attendance). Only males with tenures greater than one night are included in this figure.
All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) Version 10.0 (SPSS Inc. 1999). All tests were conducted at the $a=0.05$ level and were two-tailed unless otherwise noted.

**RESULTS**

The 1998 bullfrog chorusing season on Lake Sasajewun spanned 46 nights from 10 June 1998 to 25 July 1998, during which time 82 male bullfrogs participated in chorusing activity. The nightly number of males present ranged from nine to 50 ($X \pm SD=28 \pm 9.9$ males; Fig. 2a) and we observed 14 matings (Fig. 2b). Male bullfrogs moved many times among choruses within Lake Sasajewun, however, these movements did not affect chorus tenure and dominant tenure estimates because these measurements were summed across choruses. We censused other bodies of water infrequently during the summer and observed three males attending choruses on lakes other than the main study site. We excluded these three males from all analyses requiring chorus tenure and dominant tenure because of the likelihood that these measurements were underestimated. Chorus tenure varied among bullfrogs (Fig. 3a), as did dominant tenure (Fig. 3b). Percentage attendance (tenure/number of nights spanned by tenure) during chorus tenure was high ($X \pm SD=73.26 \pm 20.43\% ;$ median $=75\% ;$ $N=72$; Fig. 4a) as it was during dominant tenure ($X \pm SD=60.82 \pm 18.72\% ;$ median $=60\% , N=55$; Fig. 4b). Percentage of chorus tenure spent as a dominant male varied among males (median $=51.85\%$; interquartile range $30.3$–$66.7\% , N=79$). Six males had only one night of chorus tenure (Fig. 3a) and 25 males had no dominant tenure or only one night of dominant tenure (Fig. 3b). We excluded these males (the three males that chorused on other lakes) from the chorus tenure and dominant tenure multiple regressions, respectively.

**Energetic Constraint Hypothesis**

There were differences in both chorus tenure and dominant tenure among males that we observed mating more than once ($N=2$), only once ($N=9$), and that we never observed mating ($N=68$) (Kruskal–Wallis test: chorus tenure: $X^2=6.588$, $P<0.038$; dominant tenure: $X^2=8.434$, $P<0.016$; Fig. 5). Males that we observed mating only once had significantly longer chorus tenure (median=26.0 nights) and dominant tenures (median=15.0 nights) than males that we never observed mating (median chorus tenure=12.0 nights, Mann–Whitney $U$ test: $U=174.0, N_1=9, N_2=68, P<0.037$; median dominant tenure=6.0 nights, Mann–Whitney $U$ test: $U=156.5, N_1=9, N_2=68, P<0.018$; Fig. 5).

Bullfrogs participating in chorusing activity during the 1998 season lost condition during their chorus tenure (mean condition change: $X \pm SD=−8.74 \pm 13.86$ g; one-tailed $t$ test: $t_{46}=4.321, P<0.001$) and during their dominant tenure (mean condition change: $X \pm SD=−9.99 \pm 11.29$ g; one-tailed $t$ test: $t_{50}=−5.381, P<0.001$). Chorus tenure and dominant tenure were negatively correlated with change in condition, although the regression for dominant tenure was only significant at the 5.5% level (Fig. 6). The average rate of condition change during dominant tenure was significantly negative ($X \pm SD=−0.69 \pm 1.26$ g/day; one-tailed $t$ test: $t_{36}=3.351$, $P<0.003$), however, the average rate of condition change during chorus tenure was not significantly different from zero ($X \pm SD=−0.10 \pm 1.11$ g/day; one-tailed $t$ test: $t_{46}=0.591, P<0.559$). Males did not return from absences from chorus tenure in better condition than when they left (mean condition change: $X \pm SD=0.87 \pm 4.94$ g; one-sample $t$ test: $t_{27}=1.341, P<0.186$), and males that we never observed mating had significantly lower condition change after initial capture (mean condition change: $X \pm SD=−1.68 \pm 4.30$ g; one-sample $t$ test: $t_{50}=2.788, P<0.008$). Released and sham-fed treatment groups did not differ in the number of nights that individual frogs took to resume chorusing activity following their initial capture (median: released: 2 nights; sham-fed: 2 nights; Mann–Whitney $U$ test: $U=1.580, N_1=25, N_2=27, P<0.115$) or in whether frogs returned to the same chorus after initial capture (percentage returning: released: 14/25=56.0%; sham-fed: 12/27=44.4%; chi-square test: $X^2=1.476$, $P>0.50$). Therefore, these two groups were pooled as unfed frogs.
The multiple regression was statistically significant (\(r = -0.482, F_{1,45} = 13.611, P < 0.002\)) and dominant tenure (multiple regression: \(F_{1,45} = 3.986, P < 0.055\)). Only unfed males (released and sham-fed) with tenures greater than one night are included in this figure (see Methods).

Fed males were in better condition than unfed males at the end of both chorus tenure (\(X \pm SD\) final condition: fed: \(5.36 \pm 10.59\) g; unfed: \(-7.50 \pm 14.51\) g; \(t_{70} = 3.908, P < 0.001\)) and dominant tenure (\(X \pm SD\) final condition: fed: \(4.10 \pm 9.59\) g; unfed: \(-9.25 \pm 14.76\); \(t_{53} = 3.485, P < 0.002\)). However, fed males lost condition more slowly than unfed males during chorus tenure (\(X \pm SD\) rate of condition change: fed: \(1.02 \pm 1.98\) g/day; unfed: \(-0.10 \pm 1.11\) g/day; \(t\) test for unequal variances: \(t_{32.2} = 2.601, P < 0.015\)) but not dominant tenure (\(X \pm SD\) rate of condition change: fed: \(-0.13 \pm 1.40\) g/day; unfed: \(-0.69 \pm 1.26\) g/day; \(t_{53} = 1.497, P = 0.788\)).

We examined the residuals of both multiple regressions using normal probability plots and found that the assumption of normality had not been violated. The four independent variables (initial condition, final condition, rate of condition change and feeding status) explained 23.8% (19.2% adjusted) of the variation in chorus tenure (multiple regression: \(F_{4,67} = 5.220, P < 0.002\); Table 1) and explained 21.7% (15.5% adjusted) of the variation in dominant tenure (multiple regression: \(F_{4,50} = 3.471, P < 0.015\); Table 2). For the multiple regression of chorus tenure on the four predictors, only the regression coefficients of rate of condition change and feeding status were significant, and only the relationship between feeding status and chorus tenure was in the predicted direction. Other things being equal, males with longer chorus tenures had, on average, more negative rates of condition change than males with shorter chorus tenures. Only one prediction was proven correct, that fed males had longer chorus tenures than unfed males (Table 1, Fig. 7), although the median chorus tenure of fed males was lower (12 nights) than that of unfed males (14.5 nights). In the multiple regression of dominant tenure on the four predictors, the regression coefficients of initial condition, final condition and rate of condition change were highly significant and in the predicted direction. Other things being equal, males with longer dominant tenures had, on average: (1) better initial condition; (2) poorer final condition; and (3) more positive rates of condition change than males with shorter dominant tenures; but, fed males (median dominant tenure = 7 nights) did not have significantly longer dominant tenures than unfed males (median dominant tenure = 7 nights) (Table 2, Fig. 8).

**Evidence of Selection**

The selection differential and the selection gradient for initial chorus tenure condition were both significant, however, none of the selection coefficients for the other three predictors calculated for chorus tenure were significant (Table 3). The selection differential and selection gradient for initial dominant tenure condition were significant, as well as the selection gradient for final dominant tenure condition (Table 4). The selection coefficients for rate of condition change and feeding status calculated for dominant tenure were not significant (Table 4).

**DISCUSSION**

As in previous years (MacDonald 1997) and in other species of anurans (reviewed in: Murphy 1992; Halliday & Murphy 1992; Halliday & Murphy 1992).
male bullfrogs that we observed mating had longer chorus tenures than males that we never observed mating. And perhaps more consequential for sexual selection, reproductively successful males had longer dominant tenures (i.e. more nights spent calling and defending a territory) than did unsuccessful males. This is evidence that sexual selection, through endurance rivalry, may be acting on male traits that would increase male tenure (chorus tenure and dominant tenure).

Table 2. Summary of the results of the multiple regression of dominant tenure on initial condition, final condition, rate of condition change and feeding status

<table>
<thead>
<tr>
<th>Variables</th>
<th>Dominant tenure</th>
<th>IC</th>
<th>FC</th>
<th>RCC</th>
<th>B</th>
<th>β</th>
<th>pr</th>
<th>sr</th>
<th>t</th>
<th>P</th>
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<tr>
<td>Initial condition (IC)</td>
<td>0.061</td>
<td>0.369</td>
<td>0.534</td>
<td>0.357</td>
<td>0.339</td>
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<td>Final condition (FC)</td>
<td>−0.084</td>
<td>−0.318</td>
<td>−0.616</td>
<td>−0.397</td>
<td>−0.383</td>
<td>3.060</td>
<td>0.005</td>
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<td>Rate of condition change (RCC)</td>
<td>0.211</td>
<td>2.520</td>
<td>0.440</td>
<td>0.374</td>
<td>0.357</td>
<td>2.850</td>
<td>0.007</td>
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<tr>
<td>Feeding status (0=unfed, 1=fed)</td>
<td>0.173</td>
<td>3.664</td>
<td>0.229</td>
<td>0.226</td>
<td>0.205</td>
<td>1.638</td>
<td>0.109</td>
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<td>Intercept</td>
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<td>R²</td>
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<tr>
<td>Adjusted R²</td>
<td>0.155</td>
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The multiple regression was statistically significant ($r=0.466$, $F_{4,50}=3.471$, $P<0.015$) and explained 15.5% of the variation in dominant tenure. The first four columns of numbers are a matrix of one-tailed Pearson correlation coefficients ($r$). $B$ is the unstandardized regression coefficient, $\beta$ is the standardized regression coefficient, $pr$ is the partial correlation coefficient, $sr$ is the semipartial correlation coefficient and $t$ is the test statistic of the significance test for all of the regression coefficients. *$P<0.05$; **$P<0.01$.

However, the prevalence of abbreviated tenure in male bullfrogs (MacDonald 1997; Fig. 3) indicates that there are constraints on the length of time a male is able to devote to breeding site attendance.

As in other anuran studies (e.g. Wells 1978; Arak 1983; Robertson 1986; Given 1988; Ryser 1989; Murphy 1994b; MacDonald 1997), tenure was costly for male bullfrogs. Males lost a significant amount of body condition during their chorus tenure and dominant tenure. Bullfrogs lost

Figure 7. Partial regression plots of chorus tenure residuals versus the residuals of (a) initial condition ($pr^2=0.00$), (b) final condition ($pr^2=0.04$), (c) rate of condition change ($pr^2=0.09$) and (d) feeding status ($pr^2=0.06$); $pr$ is the partial regression coefficient from the multiple regression of chorus tenure versus the four predictors (see text and Table 1).
condition at an average rate of approximately 0.69 g/night of dominant tenure (the average rate of condition loss over chorus tenure was not significantly different from zero), which is much faster than the rate at which barking treefrogs lose weight (median rate of condition loss=0.125 g/night of chorus tenure, from Murphy 1994b). However, the average male bullfrog in our study was 108.3 g, whereas the average male barking treefrog in Murphy’s (1994b) study was 12.6 g. Thus, as a percentage of body mass lost per day, bullfrogs lost 0.57%/night of dominant tenure, whereas barking treefrogs lost 0.99%/night of chorus tenure. Given this result, it is striking to note that median dominant tenure of bullfrogs in our study was 7 nights, and the median chorus tenure of barking treefrogs was 2–3 nights (Murphy 1994b). Male barking treefrogs appear to devote almost twice as many resources (in terms of body mass) to their nightly chorusing effort and attend choruses for approximately one-half as many nights as male bullfrogs.

There was weak support for the energetic constraint hypothesis as an explanation for variation in chorus tenure. The four predictors of the energetic constraint hypothesis together explained 19.2% of the variation in chorus tenure, but only one of the four predictions was upheld: male bullfrogs that were fed had longer chorus tenures than males that were not fed, when the other variables were controlled (prediction 4). However, when considered alone, the median chorus tenure of fed males (12 nights) was actually shorter than the median chorus tenure of unfed males (14.5 nights). When comparing the

![Figure 8](image_url)

**Figure 8.** Partial regression plots of dominant tenure residuals versus the residuals of (a) initial condition ($p^2=0.13$), (b) final condition ($p^2=0.16$), (c) rate of condition change ($p^2=0.14$) and (d) feeding status ($p^2=0.05$); $pr$ is the partial regression coefficient from the multiple regression of dominant tenure versus the four predictors (see text and Table 2).

**Table 3.** Directional selection differentials ($s$) and gradients ($\beta\pm\text{SE}$) for male bullfrogs that attended chorusing activity on Lake Sasajewun in 1998

<table>
<thead>
<tr>
<th>Variable</th>
<th>$s$</th>
<th>$t$</th>
<th>$P$</th>
<th>$\beta\pm\text{SE}$</th>
<th>$t$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial condition</td>
<td>1.357</td>
<td>2.581</td>
<td>0.013</td>
<td>0.303±0.112</td>
<td>2.603</td>
<td>0.012</td>
</tr>
<tr>
<td>Final condition</td>
<td>−0.883</td>
<td>0.979</td>
<td>0.332</td>
<td>−0.153±0.116</td>
<td>1.266</td>
<td>0.211</td>
</tr>
<tr>
<td>Rate of condition change</td>
<td>−0.154</td>
<td>1.622</td>
<td>0.110</td>
<td>−0.026±0.118</td>
<td>0.212</td>
<td>0.834</td>
</tr>
<tr>
<td>Feeding status (0=unfed, 1=fed)</td>
<td>−0.026</td>
<td>0.883</td>
<td>0.381</td>
<td>−0.042±0.118</td>
<td>0.344</td>
<td>0.733</td>
</tr>
</tbody>
</table>

Selection coefficients were calculated for the predictors of the chorus tenure multiple regression.
The energetic constraint hypothesis was a much stronger explanation of variation in male bullfrog dominant tenure. Three of the four predictions were supported and the energetic constraint hypothesis explained 15.5% of the variation in dominant tenure. Males with longer dominant tenures, started their dominant tenure in better condition (prediction 1), allowed their condition to decline more (prediction 2) and lost condition more slowly (prediction 3) than males that had shorter dominant tenures. However, contrary to our prediction, fed males did not have longer dominant tenures than unfed males (prediction 4).

A night of dominant tenure is probably much more energetically expensive than a night of subordinate tenure because dominant males have to expend energy in calling, maintaining an inflated posture and reacting aggressively to the challenges of other males (Emlen 1976). Although dominant tenure and chorus tenure are highly positively correlated (Pearson product–moment correlation: \( r_{57} = 0.926, P < 0.001 \)), the inclusion of subordinate tenure (and its lower energetic costs) within chorus tenure probably obscured the relationships between the four predictors and chorus tenure. We were unable to analyse subordinate tenure independently because of our inability to calculate condition change for the frequent instances when subordinate tenure lasted only one night.

Better condition at the start of dominant tenure may have been the result of increased foraging success before dominant tenure began, or of better overwintering condition (indistinguishable in our study). Alternatively, males in better initial condition may simply have started dominant tenure earlier than males in poorer initial condition. If the latter was the case, then there should have been a negative relationship between the date on which males started their dominant tenure and their condition on that day. However, there was only a weak, marginally nonsignificant, negative correlation between initial and initial condition (Pearson product–moment correlation: \( r_{53} = -0.234, P < 0.087 \)), indicating that the relationship between initial condition and dominant tenure was likely real and not simply an artefact of the date on which males started their dominant tenure. MacDonald (1997) did not investigate the relationship between dominant tenure and initial condition. However, we were able to analyse the data from 1996 and there was a weak, but significant, bivariate correlation between dominant tenure and initial condition (Pearson

<table>
<thead>
<tr>
<th>Variable</th>
<th>( s )</th>
<th>( t )</th>
<th>( P )</th>
<th>( \beta \pm SE )</th>
<th>( t )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial condition</td>
<td>7.194</td>
<td>2.072</td>
<td>0.044</td>
<td>0.437±0.121</td>
<td>3.440</td>
<td>0.002</td>
</tr>
<tr>
<td>Final condition</td>
<td>-1.084</td>
<td>0.225</td>
<td>0.824</td>
<td>-0.323±0.128</td>
<td>2.417</td>
<td>0.020</td>
</tr>
<tr>
<td>Rate of condition change</td>
<td>-0.147</td>
<td>0.338</td>
<td>0.737</td>
<td>0.233±0.131</td>
<td>1.695</td>
<td>0.096</td>
</tr>
<tr>
<td>Feeding status (0=unfed, 1=fed)</td>
<td>-0.097</td>
<td>0.622</td>
<td>0.538</td>
<td>-0.082±0.134</td>
<td>-0.580</td>
<td>0.564</td>
</tr>
</tbody>
</table>

Selection coefficients were calculated for the predictors of the dominant tenure multiple regression.
product–moment correlation: $r_{102}=0.287$, $P<0.003$; R. J. Brooks, unpublished data). As described above, male barking treefrogs that started their chorus tenure in better condition had longer chorus tenures than males that started in poorer condition (Murphy 1994b).

Male bullfrogs that were in poorer final condition had longer dominant tenures than males that ended their dominant tenure in better condition. However, since bullfrogs generally lose condition during the breeding season, this result may have simply been a by-product of a correlation between the date on which males ended their dominant tenure and their condition on that date. However, this was not the case, as final condition was unrelated to the final date of dominant tenure ($r_{53}=0.006$, $P=0.969$). Final energetic condition can constrain dominant tenure in two ways: (1) there may be a lower threshold of body condition below which males cannot continue to participate in breeding activity (i.e. a current cost); and (2) decreased body condition may be costly in terms of overwinter survival and future reproduction (i.e. a future cost).

If there were a lower limit of body condition, then males should have always returned to the chorus after a temporary absence (Bevier 1997; Green 1990; Murphy 1994a; MacDonald 1997) in better condition than when they left (Bevier 1997). However, male bullfrogs did not increase their body condition while they were absent from dominant tenure, and in fact returned in poorer condition than when they left, indicating that they probably did not forage. Therefore, male bullfrogs did not temporarily leave chorusing activity because their energetic condition was too low to allow them to continue to participate. However, the reason that male bullfrogs temporarily left chorus activity is still unknown. Male bullfrog body condition may interact with a number of variables such as: environmental conditions (temperature, wind velocity, etc.), the operational sex ratio, risk of predation, individual age and overall vigour, to cause some individuals to leave and others to remain active (Lucas & Howard 1995; Lucas et al. 1996). In the future, it would be interesting to use radiotelemetry to find out what male bullfrogs do when they are not participating in chorus activity.

Decreased body condition may be costly in terms of overwinter survival and future reproduction. If this were the case, then males that survived to the following winter should have had a higher mean final condition than males that did not survive, and/or among overwinter survivors, final condition in 1998 should have been positively related to initial condition in 1999. Males that survived to 1999 did not have significantly higher final condition than males that did not survive ($X \pm SD$: survivors: $-4.01 \pm 13.15$ g; nonsurvivors: $-2.80 \pm 14.99$ g; $t_{n}=0.719$, $P<0.783$). However, for bullfrogs that did survive to 1999, there was a significant positive correlation between final condition in 1998 and initial condition in 1999 (one-tailed Pearson product–moment correlation: $r_{4}=0.456$, $P<0.039$). This means that among overwinter survivors, bullfrogs that chorused longer in 1998 paid an energetic cost in terms of poorer condition at the start of the 1999 season, relative to males that put less effort into chorusing in 1998. Given the positive relationship shown between initial condition and dominant tenure in 1998, this is evidence of a trade-off between current and future chorusing effort. If the probability of survival decreases with age, then older males are predicted to spend more time attending choruses (Lucas et al. 1996). MacDonald (1997) found that veteran male bullfrogs chorused longer than first-year males. However, in our study there were no differences among three veteran classes (first-year, second-year and third-year chorusers) in dominant tenure (Kruskal–Wallis test: $\chi^2=0.163$, $P<0.923$).

Male bullfrogs that lost body condition more slowly had longer dominant tenures than males that lost body condition more rapidly (Table 2, Fig. 8). In the only other study to examine this relationship, Murphy (1994b) also found that the rate of condition change in male barking treefrogs was positively related to chorus tenure.

Our feeding experiment did not have a significant effect on dominant tenure (Table 2, Fig. 8, median dominant tenure for both fed and unfed males=7 nights). Previous feeding experiments have shown mixed results in causing an increase in chorus tenure. Male barking treefrogs significantly increased their chorus tenures when fed (Murphy 1994b). Fed and starved male túngara frogs, Physalaemus pustulosus, showed no significant differences in chorus tenure, call rate, or call complexity (Green 1990), but in a later study (Marler & Ryan 1996), fed males were significantly more likely to call than unfed males (but see Marler & Ryan 1996 for a discussion of the difficulties in comparing these two studies). Per meal, we fed bullfrogs approximately 0.87 kJ/g body mass (mean bullfrog body mass=108.3 g), which was much more than the 0.50 kJ/g body mass/meal (mean barking treefrog mass=12.6 g) fed to male barking treefrogs (calculated from Murphy 1994b) or the 0.41 kJ/g body mass/meal (mean túngara frog body mass=1.7 g) fed to male túngara frogs (calculated from Green 1990; meal size was not given in Marler & Ryan 1996). Because we do not know the amount of energy used by male bullfrogs in one night of calling, we cannot calculate the value (in number of nights of calling) of a meal of two chicken hearts, as used in the present study. Therefore, the energy value of the meal that our fed males received may have been too small to cause an increase in dominant tenure. We did find that fed males ended their dominant tenures in better condition than unfed males. This suggests that the feeding treatment did provide males with a significant amount of energy, but the males directed this energy into fat reserves or growth instead of increasing their dominant tenures.

In addition to increasing the number of nights spent chorusing, fed male bullfrogs could have increased their chorusing effort at a different time scale (e.g. the amount of time that they chorused within a night and/or their call rate). Data on these finer scales of bullfrog chorusing effort have not yet been gathered for this study system, and may provide very different insights into male bullfrog reproductive effort than chorus tenure and dominant tenure. For example, Murphy (1994b) found that chorus tenure increased significantly when male barking
Evidence of Selection

Our analysis of directional selection on the four predictors of the energetic constraint hypothesis revealed strong directional selection favouring better (more positive) initial condition. This was the case whether we analysed initial chorus tenure condition (Table 3) or initial dominant tenure condition (Table 4). One or all of three mechanisms of sexual selection are likely to be responsible for the direct selection on initial condition. Most aggressive encounters between male bullfrogs occur early in the breeding season (Howard 1984; personal observation). Because larger body size confers a significant advantage to male bullfrogs in aggressive encounters (Howard 1984), and because large males control high-quality territories that females prefer (Howard 1978b), it is likely that better initial body condition is favoured by direct male–male competition. Male body condition is also likely to be related to male quality, and as such, higher initial condition is probably preferred by female bullfrogs, either because of direct genetic benefits or because of increased fertilization rates (e.g. Ryan 1983, 1985). Given that increased initial body condition resulted in longer dominant tenure (Table 2, Fig. 8), endurance rivalry probably selected directly for initial dominant tenure condition. Endurance rivalry could not have been responsible for the direct selection on initial chorus tenure condition because the energetic constraint hypothesis did not adequately predict variation in chorus tenure (Table 1, Fig. 7). However, the positive correlation between initial chorus tenure condition and initial dominant tenure condition ($r_{s}=0.532, P<0.001$) means that endurance rivalry could have contributed to the selection indirectly.

Selection on final condition provides an example of the difference between selection differentials and selection gradients. There was no significant directional selection differential on final condition indicating that there was no significant change in mean final condition as a result of selection (Table 4). However, the selection gradient was significant (Table 4), which means that there was significant directional selection (negative) directly on final condition, but that it was masked by indirect selection through some correlated male trait(s). Direct selection to increase initial condition (which was positively correlated with final condition, Table 2) probably produced positive indirect selection on final condition, counteracting the direct negative selection on final condition. This is an excellent example of the utility of calculating directional selection differentials and gradients (Lande & Arnold 1983).

Negative selection on final condition means that male bullfrogs that increase the cost of their dominant tenures by allowing their condition to decline more, are more reproductively successful than males that allow their condition to decline less. Energetically costly displays, such as frequent bouts of wrestling or high call rates, may be preferred by females because they indicate the high quality of males that are able to overcome the handicap of producing the display (Zahavi 1975). Alternatively, the negative relationship between dominant tenure and final condition is evidence that final condition is important to endurance rivalry.

Although we cannot determine which of the three mechanisms: male–male competition, female choice or endurance rivalry, is responsible for the directional selection on initial condition and final condition, ours is the first study to explicitly identify male traits that could have been selected by endurance rivalry.

Acknowledgments

We thank S. Swanson, N. Boyd, K. McTaggart, L. Snow and L. Sachro for their excellent assistance in the field. R. McLaughlin and T. Nudds were very helpful in the planning of this project and provided constructive criticism of early drafts of this manuscript. We would like to thank J. Lucas, K. Wells and one anonymous referee for their valuable comments on this manuscript. C. Murphy also provided stimulating discussion and important insights regarding the ideas presented here. The staff at the Wildlife Research Station made conducting research in the wilds of Algonquin Provincial Park a pleasure. Research was supported by a Natural Sciences and Engineering Research Council Post Graduate Scholarship to K.A.J. and a NSERC Operating Grant to R.J.B. The research presented here was described in Animal Utilization Proposal No. 98R072, approved on 8 June 1998 by the University of Guelph Animal Care Committee. This research was further evaluated and approved by the Animal Behavior Society’s Animal Care Committee on 14 June 2001.

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