In many animals, sexual selection on male traits results from female mate choice decisions made during a sequence of courtship behaviors. We use a bower-building cichlid fish, *Nyassachromis cf. microcephalus*, to show how applying standard selection analysis to data on sequential female assessment provides new insights into sexual selection by mate choice. We first show that the cumulative selection differentials confirm previous results suggesting female choice favors males holding large volcano-shaped sand bowers. The sequential assessment analysis reveals these cumulative differentials are the result of selection acting on different bower dimensions during the courtship sequence; females choose to follow males courting from tall bowers, but choose to engage in premating circling with males holding bowers with large diameter platforms. The approach we present extends standard selection analysis by partitioning the variances of increasingly accurate estimates of male reproductive fitness and is applicable to systems in which sequential female assessment drives sexual selection on male traits.

**Key Words:** Cichlids, extended phenotype, Lake Malawi, leks, mate choice, selection analysis.
based on the number of bone ornaments surrounding their bowers, but choose to engage in courtship behavior based on the number of glass ornaments within the bower (Borgia 1995). Female treefrogs may choose to approach males based on the acoustic properties of their call, but subsequently use visual cues to choose between courting males (Taylor et al. 2007), and female fish may use olfactory cues to refine choices based on visual signals (Hankison and Morris 2003).

Conceptually, sequential female assessment can be viewed as sequential episodes of selection. Each choice a female makes during the courtship sequence is a selection episode, and the numbers of females a male induces to proceed to each stage of the sequence represent increasingly accurate estimates of male reproductive fitness. How female choice affects the cumulative force of sexual selection on male traits depends on selection at each stage, and on how these episodes of selection change the covariance structure among correlated traits through the courtship sequence (Arnold and Wade 1984a). Just as a full understanding of trait evolution requires quantifying episodes of natural and sexual selection during different life-history stages, a full understanding of how female mate choice drives evolution in male traits requires quantifying selection at different stages of the courtship sequence.

Here, we use a bower-building cichlid fish to demonstrate how applying selection analysis to sequential assessment data provides new insights into how mate choice drives sexual selection on male traits.

Material and Methods

STUDY SYSTEM

We studied *Nyassachromis cf. microcephalus* (Trewavas 1935; Konings 2007) on a large lek in southern Lake Malawi, Africa (Fig. 1A). The species is a small (maximum total length 10–13 cm) planktivore that forms breeding leks in near-shore sand habitats (see Konings 2007 for further information). Males construct, maintain, and defend volcano-shaped sand bowers from which they court passing females (Fig. 1B). Bowers can be considered as extended phenotypic signals used by females during mate choice decisions and/or by conspecific males during territorial disputes (Genner et al. 2008; Schaedelin and Taborsky 2009). Previous work on Lake Malawi bower-building cichlids suggests male reproductive fitness increases with the quality (bower size) and position (centrality) of these extended phenotypes (McKaye et al. 1990; Kellogg et al. 2000; Stauffer et al. 2005; Genner et al. 2008). Two features of the system make it well suited for studying how sequential female assessment drives sexual selection. First, the courtship sequence involves an invariant sequence of discrete, easily identified behaviors. Second, males reliably court females that pass their territory (Young et al. 2009), but do not search for, harass, or coerce females, meaning female choice determines progression through the courtship sequence.

BEHAVIORAL OBSERVATIONS AND BOWER MEASUREMENTS

We (all authors) conducted behavioral observations using SCUBA during the daily peak in breeding activity (0800–1100) 2–7 September, 2005. Orthogonal axes were used for orientation and subsequent mapping of the lek (Fig. 1A). Each observer selected males haphazardly while moving inward or outward through the lek to ensure we observed males from throughout the lek. We recognize that humans may be more likely to select “active males” for observation, which would result in a nonrandom sample of the population. We observed 50 males, each for a single 10-min period (Kellogg et al. 2000), after which we marked their bowers with numbered flags. We recorded the number of times each male engaged in the following behaviors: antagonistic interaction...
with a conspecific male, antagonistic interaction with a heterospecific individual, bower-building activity (moving sand by mouth onto the bower), foraging in the water column, and courtship of a passing female.

For each courtship event, we recorded the stage of the courtship sequence to which the female chose to proceed. The courtship sequence consists of five behaviors: (1) courtship—the male swims rapidly to the female, presents a quivering lateral display, and attempts to lead the female to the bower, (2) follow—the female chooses to follow the male to the bower, (3) visit—the female chooses to visit the bower platform, (4) circle—the female chooses to engage in premating circling with the male on the bower platform, (5) mating—the female chooses to lay one or more eggs that she collects in her mouth as they are fertilized by the male. Mating was rare during our observations. We observed only two mating events during 500 min of observations. Fortunately, each stage of the courtship sequence provides an increasingly accurate estimate of number of eggs a male fertilizes (Kellogg et al. 2000; Genner et al. 2008). In our analyses, each male is characterized by four increasingly accurate measures of reproductive fitness: the number of courtships, follows, visits, and circles. The number of courtship events engaged in by a male is different from the subsequent three estimates of male fitness in that it is not clearly dependent on female choice.

Following behavioral observations, we recorded the location of every bower (Fig. 1A). For bowers associated with behavioral observations, we measured twice (at 90° angles) six bower dimensions (Kellogg et al. 2000). Based on previous research and preliminary analysis, we included four “traits” of the extended phenotype in our analysis: maximum base diameter, maximum height, maximum platform diameter (Fig. 1B), and bower position. We calculated two positions (scaled 0 to 1) for each male’s bower: the distance from the “geographical center” of the lek, where the origin (0, 0) is defined as the mean position of all x–y coordinates; and the distance from the “density center” of the lek, where the origin is defined as the mean value of all x–y coordinates. The two distance measures were highly correlated (\( r_p = 0.86 \)) and yielded qualitatively similar results. We present the results from analyses using geographical distance.

**DATA ANALYSIS**

For all analyses, bower variables were standardized \((z', \text{mean} = 0, \text{standard deviation} = 1)\) by subtracting the mean and dividing by the standard deviation. Measures of absolute male fitness (numbers of courtships, follows, etc.) were transformed to relative fitness \((w)\) by dividing each individual’s absolute fitness by mean absolute fitness (Lande and Arnold 1983; Arnold and Wade 1984a). Measures of relative fitness were left untransformed, which yields accurate selection indices but requires using nonparametric statistical tests when data are nonnormally distributed (Lande and Arnold 1983). Following standard procedure, the opportunity for selection \((I)\) was calculated as the variance in relative fitness. Standardized directional selection differentials \((\beta')\) were calculated as the covariance between relative fitness and standardized trait values. Standardized linear selection gradients \((\beta')\) were calculated as the coefficients from the multiple regression of relative fitness on standardized traits (Lande and Arnold 1983). Selection differentials quantify the combined effects of direct selection on the trait and indirect selection on measured and unmeasured correlated traits. Their values do not depend on traits (ex)cluded in the analysis. Selection gradients describe the force of selection on each trait controlling for variation in other traits (Lande and Arnold 1983). Their values do depend on the variables (ex)cluded in the regression model. Quadratic and product (height \(\times\) height, height \(\times\) base, etc.) selection differentials/gradients did not approach significance and were not included the final analyses. Our preliminary analyses found no evidence that other measured male behaviors (intra- and interspecific aggression, building and feeding) affected female choice, so these behaviors were not included in the final analyses. We provide correlation matrices of all bower measurements, all male behaviors, and the increasingly accurate measures of absolute fitness as Supporting Information.

Two modifications to standard selection analysis are appropriate when quantifying sexual selection by sequential female assessment (Arnold and Wade 1984a,b). First, males with zero fitness at any stage of the courtship sequence should be excluded from the analyses of selection at subsequent stages. Biologically, a male for which no female chooses to proceed to stage \(k\) cannot provide information about sexual selection resulting from the female choice to proceed from stage \(k\) to \(k + 1\). Practically, this requires that initial sample sizes be large enough to accommodate sequential culling. Analytically, it requires that trait variables are restandardized and relative fitness is recalculated at each stage. Standard selection analysis corrects for individuals with zero fitness by weighting fitness at episode \(k\) by fitness at episode \(k - 1\) (Arnold and Wade 1984b). Sequentially culling and recalculating has two advantages. First, it yields accurate stage-specific selection differentials/gradients because the correct variance–covariance structure of the data is used at each stage (Arnold and Wade 1984a). Second, it ensures the correct degrees of freedom are used for testing the statistical significance of selection differentials/gradients. In general, however, stage-specific selection indices calculated from culled data will not sum to match exactly the cumulative opportunities, differentials, or gradients because the covariance structure among the correlated traits typically changes through episodes of selection (Arnold and Wade 1984a).
The second modification is required by the first and involves partitioning the variance in relative fitness \( I \) at each stage of the analysis. When analyzing culled sequential assessment data, \( I_k \) does not reflect the opportunity for selection by female choice at stage \( k \) because for each male the number of females that chose to proceed to stage \( k - 1 \) affects the number that can choose to proceed to stage \( k \). The solution is to regress \( w_k \) on \( w_{k-1} \) and retain the residuals. The opportunity for selection by female choice to proceed from stage \( k - 1 \) to stage \( k \) is the variance of these residuals, which are used to calculate the selection differentials and gradients at stage \( k \).

The analysis proceeds as follows. For the first observed stage \( k \) calculate the relative fitness and standardized traits using all observed males. Calculate the opportunity for selection and the selection differentials and gradients as normal. Remove all males with \( w_k = 0 \) and recalculate the relative fitness at stage \( k \). Calculate the standardized trait values and relative fitness at stage \( k + 1 \). Regress \( w_{k+1} \) on \( w_k \), retaining the residuals. Use these residuals to calculate the opportunity for selection, and the selection differentials and gradients associated with female choice to proceed from stage \( k \) to \( k + 1 \). Repeat this procedure at each stage. The significance of selection differentials (covariances) can be tested using Spearman or Pearson correlation coefficients depending on the distribution of the data (Lande and Arnold 1983). For selection gradients significance, values from the multiple regressions can be used if strict tests of significance are of interest.

### Results

Of the 50 observed males, 46 courted at least one female, 36 had at least one female choose to follow, 30 had at least one female choose to visit the bower, and 15 had at least one female choose to engage in premating circling. We first describe the cumulative standardized selection differentials and gradients calculated using all 50 males and the most accurate measure of male reproductive fitness—the number of females that engaged in premating circling. The cumulative differentials reveal sexual selection favors males occupying tall bowers with large platforms, but that base diameter is selectively neutral (Table 1). The cumulative gradients suggest this pattern is the result of variable direct selection acting on the three bower dimensions (Table 1). Sexual selection strongly favors platform diameter; an increase in platform diameter by one standard deviation increases relative fitness by 86%. A comparable increase in bower height increases relative fitness by 43%. In contrast, controlling for bower height and platform diameter, sexual selection favors males holding bowers with small base diameters, that is, those that maximize bower height or platform diameter for a given base diameter. The gradients do not match the differentials because the positive covariances among the three bower dimensions render base diameter selectively neutral and reduce the magnitudes for bower height and platform diameter (Table 1).

The sequential analysis reveals that the cumulative forces of sexual selection are the result of stage-specific variation in how female choice drives selection on different bower dimensions (Table 1). The sequential gradients suggest bower height is favored by direct selection principally through the choice by courted females to follow a male to his bower, whereas platform diameter is under strong direct selection through the female choice to engage in premating circling. Controlling for variation in bower height and platform diameter, base diameter is under negative direct selection throughout the courtship sequence, particularly through the choice by females that have visited a bower to engage in premating circling. Figure 2 shows the cumulative and sequential selection differentials in the standardized bower height/platform diameter space (Fig. 2A). In Figure 2B, the solid arrow connecting the solid circles shows how the cumulative selection differentials act on the two bower dimensions. The dashed arrows connecting the open circles show how the sequential selection differentials act on the two bower dimensions at each of the four stages of the courtship sequence. These sequential differentials clarify how sexual selection acts on bower height and platform diameter differently through the courtship sequence. They sum to accurately predict the cumulative differential because the covariance structure among the three bower dimensions is changed little by culling males with zero fitness at each stage of the courtship sequence (shown in the bottom row of Table 1).

### Discussion

The sequential selection differentials show bower height is favored only through the choice by courted females to follow males to the bower. Although previous studies have shown bower height is correlated with estimates of male fitness, our sequential analysis provides the strongest evidence to date that this bower dimension is favored by sexual selection through direct female mate choice (Wiley and Poston 1996). McKay et al. (1990) observed male *Cyrtocara eucinostomus* on 43 pairs of short and tall bowers and found males on tall bowers courted more females, but that the proportions of courted females that chose to visit and circle in short and tall bowers were similar (data in their Fig. 2). Similarly, Kellogg et al. (2000) studying *Lethrinops cf. parvidens*, Genner
et al. (2008) studying Hemitilapia oxyrhynchus, and Martin and Genner (2009) studying N. cf. microcephalus, found that male courtship frequency increased with bower height, but that subsequent measures of male reproductive fitness did not depend on bower dimensions. Stauffer et al. (2005) used artificial bowers to experimentally demonstrate that male L. cf. parvidens with taller bowers fertilized more eggs, but their data did not identify at which stage of the courtship sequence bower height affected female choice and male fitness. Identifying the mechanisms responsible for the commonly observed positive correlations between bower height, courtship frequency, and mating success will require determining whether females adjust their search patterns based on bower height (i.e., direct mate choice), or if males on tall bowers court passing females more often than males on short bowers.

The sequential selection differentials reveal that platform diameter is favored by selection principally through the female choice to engage in premating circling. Given the physical constraints on the shape of bowers, selection acting on height and platform diameter must impose a trade-off during bower construction/maintenance; for a given base diameter and volume of sand, a male cannot maximize both bower height and platform diameter.

The relative magnitudes of the cumulative selection differentials suggest males should prioritize diameter over height. McKay et al. (1990) provided empirical support for this prediction by experimentally removing bowers and recording their dimensions during reconstruction. Mean platform diameter reached its maximum (equal to control bowers) after two days. Bower height, however, continued to increase and had not reached control levels after two weeks. Schaedelin and Taborsky (2006) documented a similarly rapid (one day) return to predestruction diameters in the field and laboratory using the Lake Tanganyika bower building cichlid Cyathopharynx fucifer.

The selection gradients and differentials from the sequential analysis suggest male courtship rate is negatively related to bower height and platform diameter (Table 1; Fig. 2B). This result is in contrast to previous studies and highlights the fact that the result is in contrast to previous studies and highlights the fact that using sequential assessment data to quantify selection through direct female choice at any stage of a courtship sequence requires controlling for the variance in male relative fitness at the previous stage. In this system, data from focal male observations cannot be used to test if direct female choice imposes selection at the courtship stage because courtship rate is the first measure of male fitness and there is no information on female behavior prior to...
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Figure 2. (A) The distribution of the 50 bowers in standardized (mean = 0, SD = 1) bower height/platform diameter space. (B) Detail of the center of (A) showing the standardized selection differentials on platform diameter and height. The two filled circles and solid arrow show the cumulative selection differentials using all males and the single most accurate estimate of male reproductive fitness—the number of females that engage in premating circling. The open circles and dashed lines show the sequential selection differentials associated with the four stages of the courtship sequence: number of courtships, female follows, bower visits, circling events.

courtship. The negative selection indices for courtship rate are artifacts of this limitation. Males with short, small-diameter bowers may be observed to court more females because males holding tall, large-diameter platforms are favored by direct female choice, more frequently engage in later stages of the courtship sequence, and thus court fewer of the females that pass their territories (Young et al. 2009).

Selection analysis does not provide predictions about trait evolution (Arnold and Wade 1984a). The evolutionary consequences of the selective forces documented here depend on the degree to which bowers are genetically fixed or condition-dependent extended phenotypes of males. We know of no data testing if intraspecific variation in bower shape has a heritable basis. However, a number of observations suggest bowers can be considered as extended phenotypic territories of varying quality whose acquisition, maintenance, and defense depend on male condition and competitive ability. First, male turnover on leks is common, so multiple males hold the same bower territory during a single breeding season (Kellogg et al. 2000; Schaedelin and Taborsky 2006). Second, males holding large bowers expend more energy on territory defense (McKay et al. 1990). Third, platform diameter is correlated with male size, and when diameter is experimentally enlarged or reduced males rapidly rebuild bowers to the original size (Schaedelin and Taborsky 2006). This suggests males adjust their bowers to match their condition, similar to the pattern observed following the experimental manipulation of bower quality in the spotted bower bird *Chlamydera maculata* (Madden 2002). Consistent with the idea that bower height is used as an indicator of condition or competitive ability, Martin and Genner (2009) used artificial bowers to experimentally demonstrate that male *N. cf. microcephalus* were more aggressive toward invading males on short than tall bowers. Finally, although we did not find evidence that recorded behaviors other than courtship frequency affected male fitness, we suspect that females use other (condition-dependent) male signals and behaviors such as color, odor, and display intensity during mate choice.

Viewing bowers as territories whose size and location accurately reflect male condition and competitive ability has two implications. First, when female mate preference and male–male competition impose similar selection pressures on male traits, male–male competition will facilitate accurate female choice, direct and indirect mate choice will act in concert, and their evolutionary consequence will be similar (Candolin 1999; Sæther et al. 2005; Wong and Candolin 2005). Second, the evolutionary consequences will depend on the origin of variation in male condition. If there is high genetic variance in condition, as is expected because condition will typically depend on the combined effects of multiple genes (Houle 1991; Price and Schluter 1991), sexual selection on bower size will act on and capture that variance, favoring the “good genes” responsible for condition (Rowe and Houle 1996). But males holding territories on leks forego foraging opportunities and engage in costly behaviors to maintain, enhance, and defend their bowers (McKay et al. 1990; Bucher 2004; Young et al. 2009). Male condition should thus decline through time until they are forced onto inferior bowers or off the lek by newly arriving males in better condition (J. Stauffer, pers. comm.). If accompanied by a decline in reproductive success, the temporal decline in individual condition may act to reduce between individual variation in condition and reproductive fitness. Both the genetic capture and declining condition scenarios would act to maintain genetic variation in the face of directional selection on bower size. Distinguishing between the two hypotheses and identifying the role of other male behaviors/traits in mate choice will require marking and observing individual males and bowers.
throughout the breeding season (Kellogg et al. 2000). These data will also clarify the dynamic relationship between bower size, female choice, and male condition, residency, and competitive ability.

We have shown how the framework designed to quantify sequential episodes of selection is easily modified to analyze selection by sequential female assessment during a single episode of sexual selection. The approach is broadly applicable to systems in which female choice determines the progression through a series of discrete courtship behaviors. We suspect many researchers studying such systems already have data amenable to the analytical approach presented here. Our approach follows directly from the original framework based on the partitioning of variances and covariances (Arnold and Wade 1984a,b). It can thus easily accommodate more complex General Linear Models to provide insights into how temporal and spatial variation in demographic and environmental conditions affects patterns of sexual selection by sequential female assessment (e.g., Kasumovic et al. 2008; Reichard et al. 2009).

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LITERATURE CITED
SEXUAL SELECTION IN BOWER-BUILDING CICHLIDS


Supporting Information
The following supporting information is available for this article:

Table S1. Pearson correlation coefficients between bower position and dimensions, the five recorded male behaviors including courtship, and the three subsequent stages of the courtship sequence that depend on female choice. These coefficients are based on data from observations of all 50 males.
Table S2. Pearson correlation coefficients between bower position and dimensions, the five recorded male behaviors including courtship, and the three subsequent stages of the courtship sequence that depend on female choice. These coefficients are based on data from observations of the 46 males that courted at least one female.
Table S3. Pearson correlation coefficients between bower position and dimensions, the five recorded male behaviors including courtship, and the three subsequent stages of the courtship sequence that depend on female choice. These coefficients are based on data from observations of the 36 males that induced at least one courted female to follow them to their bower.
Table S4. Pearson correlation coefficients between bower position and dimensions, the five recorded male behaviors including courtship, and the three subsequent stages of the courtship sequence that depend on female choice. These coefficients are based on data from observations of the 30 males for which at least one following female visited the bower.

Supporting Information may be found in the online version of this article.

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