

Tests of hypotheses concerning early male arrival based on studies of two species of passerines: Ruby-crowned Kinglets (*Regulus calendula*) and Song Sparrows (*Melospiza m. melodia*).

By

Darryl B. Edwards

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ABSTRACT

Earlier male arrival at breeding sites is believed to be a sexually selected trait in passerine birds. Consistent with this hypothesis, I found that males with brighter crests, arrived earlier than males with duller crests in Ruby-crowned Kinglets (*Regulus calendula*): a species possessing sexually dimorphic plumage and arrival times. This result, although consistent with selection occurring in the competing sex, could simply reflect condition-dependent arrival. Migration is energetically costly, yet neither fat load nor residual mass predicted arrival in either sex in Ruby-crowned Kinglets. In a second study, I tested the prediction that sexually monomorphic species, such as Song Sparrows (*Melospiza m. melodia*), would show reduced protandry. Consistent with this prediction, I found that males did not precede females at breeding sites in spring. There was also evidence of condition-dependent arrival in both sexes. If intra-sexual selection advances arrival dates in both sexes, then degree of protandry would be reduced as a consequence.

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TABLE OF CONTENTS

	Page
Abstract.....	ii
Acknowledgements.....	iii
Table of Contents.....	v
List of Figures.....	vii
General Introduction.....	1
Chapter 1. Migratory timing is predicted by plumage coloration in male Ruby-crowned Kinglets (<i>Regulus calendula</i>).....	7
Introduction.....	7
Materials and Methods.....	9
Study Site.....	9
Study Species.....	9
Measurements.....	11
Colorimetric Analysis.....	11
Ptilochronology.....	12
Statistical Analysis.....	13
Results.....	14
Discussion.....	16
Chapter 2. Concomitant selection for early arrival of females may reduce the degree of protandry in the monogamous Song Sparrows (<i>Melospiza melodia</i>).....	22

TABLE OF CONTENTS
(continued)

Introduction.....	22
Study Species.....	23
Materials and Methods.....	24
Study Site.....	24
Sampling Period.....	25
Field Protocol.....	25
Statistical Analysis.....	27
Results.....	28
Sample and Genetic Sexing Results.....	28
Differential Arrival.....	29
Sexual Size Dimorphism.....	29
Intra-sexual Arrival.....	29
Discussion.....	33
Summary and Conclusions.....	40
Literature Cited.....	43
Appendix I. Errors in sexing passerines using reproductive characters: a case study with <i>Melospiza m. melodia</i>	55
Introduction.....	55
Materials and Methods.....	57
Results.....	60
Discussion.....	62

LIST OF FIGURES

Figure	Page
1.1 Study Site: Ruthven Park, Haldimand Bird Observatory.....	10
1.2 Crest Colour relative to arrival date in male Ruby-crowned Kinglets.....	15
2.1 Females Song Sparrows that arrived on the breeding grounds earlier were larger	31
2.2 Fat score at time of capture in male Song Sparrows.....	32

GENERAL INTRODUCTION

For many passerines, migration is a significant circannual event. Over half of North American species undergo a long-distance migration. The distance travelled between the breeding grounds and the wintering grounds can be measured in thousands of kilometres and the voyage requires a major time and energy investment. That migration is energetically costly has been well documented. Butler and Woakes (1990) report that energy expenditure in migrating passerines can be as much as 2.3 times higher than mammals undertaking maximum exercise. The strain of migratory flight coupled with periods of fasting can result in dehydration and the loss of muscle and lipids (Jenni and Jenni-Eiermann 1998). For example, Bar-tailed Godwits can lose approximately one quarter of their muscle mass and organ tissue during their long migration to the breeding grounds (Piersma and Jukema 1990). Reductions in hematocrit and immune function also likely accompany changes in energetic condition (Merila and Svensson 1995).

The extended bouts of aerobic activity experienced by migrating birds necessitates efficient energy use, and lipids are considered the most suitable energy source to meet these demands (Jenni and Jenni-Eiermann 1998). Fat stores can be a critical predictor of the frequency and duration of restorative stops en route (Fransson 1998, Schaub and Jenni 2001), ultimately determining migratory rate and hence arrival date.

Given the energetic demands of migration, it is easy to implicate natural selection as a determinant of patterns of migration. However since birds are migrating to and from the breeding grounds, aspects of migration may be sexually selected if they confer a reproductive advantage. Indeed, the breeding season may be short and a timely start to

breeding can be a crucial determinant of reproductive success. Additionally breeding resources are often limiting or vary in quality. For these reasons early breeders tend to achieve higher reproductive success resulting in directional selection for breeding onset (Price *et al.* 1988). To a migratory passerine, this imposes selection on arrival dates.

Protandry, or early male arrival (EMA), is thought to be a result of sexual selection for arrival time (Rubolini *et al.* 2004). Consistent with this, the sex experiencing competition for breeding resources arrives on the breeding grounds first – most often males but the premise also holds in instances of sex-role reversal. Further males of species subjected to the highest levels of competition arrive earlier relative to females (Rubolini *et al.* 2004). Perhaps to facilitate early arrival, males of many species overwinter closer to the breeding grounds but this is not a necessary condition to generate the pattern of EMA (Cristol *et al.* 1999). Selection for arrival time is a function of the benefits to early arrival (improved reproductive success) but is opposed by costs to migration which can affect rate and departure, as well as mortality on the breeding grounds due to harsher conditions earlier in the season (Brown and Brown 2000). The result is that not all individuals can arrive early.

There are at least two manifestations of intra-sexual competition for early arrival. Birds in poor energetic condition may delay their departure from the wintering grounds or remain at staging areas longer to increase energy stores while those in better energetic condition could initiate migration and ultimately arrive on the breeding grounds earlier. The expected result is that within a sex, individuals in better condition arrive earlier (Kokko 1999). Alternatively, as larger body size is metabolically more efficient at lower temperatures and may allow for longer fasting (Cristol *et al.* 1999), while also offering a

competitive advantage for resources (Richner 1989) as larger individuals may have a survival advantage that results in the ability to migrate earlier.

In Chapter 1, I tested for evidence for predictors of arrival date, energetic condition, size and condition-dependent traits such as plumage characters for example, in Ruby-crowned Kinglets (*Regulus calendula*). Kinglets were chosen as a model for two reasons: i) Ruby-crowned Kinglets, named for the bright red crest in males, exhibit a sexually dimorphic trait. Generally, plumage dimorphisms are considered features of female choice, are often correlated with a suite of other sexually dimorphic traits and ultimately are associated with an increased level of intra-sexual competition (Rubolini *et al.* 2004). Furthermore, since males and females differ in no other visible plumage character, assessment of the trait, in terms of size and brightness, is straightforward compared to more complex traits. ii) Ruby-crowneds have been observed to have a high degree of protandry compared with other species of dimorphic passerines (Kissner *et al.* 2003).

Consistently, the sex that competes for limited breeding resources (i.e. territories, females, etc) precedes the other in arriving on the breeding grounds (Cristol *et al.* 1999). Usually, this is males but when females compete for males the opposite is true. Males arriving on the breeding grounds early relative to other males have been shown to hold better territories, have an increased likelihood of acquiring a mate [or more mates] and have higher recruitment and an increased chance of multiple broods (Møller 1994). Thus early arriving individuals typically have a higher reproductive output suggesting that arrival date should be sexually selected. When selection for arrival occurs within a sex – individuals of one sex gain an advantage in arriving early relative to others of the same

sex, rather than of the opposite sex – this creates a situation where mean arrival date of that sex is advanced relative to the other (Kokko 1999). In other words, intra-sexual competition for breeding resources in males selects for early arrival, advancing male arrival dates relative to those of females causing protandry. For these reasons (apparent sexual dimorphism and protandry), Ruby-crowned Kinglets are a good candidate species to look for evidence of intra-sexual competition for arrival dates.

Perhaps partly due to the prediction that sexual dimorphisms and protandry are a function of the same competition, and that they are easily sexed, most studies comparing protandry across species limit their scope to those that are clearly sexually dimorphic (e.g. Kissner *et al.* 2003, Rubolini *et al.* 2004). This leaves a potentially interesting group of species that has remained relatively unstudied in this regard. Little, or no, empirical data are available for the degree of early male arrival in sexually monomorphic species (i.e. species that cannot be differentiated visually by physical characters). Reports exist of protandry occurring in monomorphic species, but the reports are largely observational anecdotes stating that males arrived before females. This type of reporting for monomorphic species means that there can be made no direct comparison with current studies using dimorphic species that report protandry as the mean difference between male and female arrival dates. Kissner *et al.* (2003) report mean male - mean female arrival differences for thirty species ranging from 0.5-12.7 days. To what degree do monomorphic species demonstrate protandry, if in fact they do? Observational studies also rely on having an equal likelihood of observing both sexes. But where males expose themselves to sing, females also may be present early in the season yet go undetected because they are secretive and cryptic. To exacerbate the problem, female Song Sparrows

are known to sing from exposed perches and aggressively chase other birds (Arcese *et al.* 2002), so estimates may be further biased when sex is assigned on the basis of 'typically' male behaviours.

Observational studies reporting protandry in monomorphic species, to large degrees in some cases (Bédard and LaPointe 1984), contradict the idea that protandry should be limited in species with reduced sexual size dimorphism and dichromatism, and limited polygyny. In Chapter 2, I tested to what degree protandry exists in a monomorphic species. I used Song Sparrows (*Melospiza m. melodia*) as my model for a variety of reasons: i) albeit largely in sedentary populations, the life history of Song Sparrows is well studied; ii) since banding at our study site has occurred in previous years, re-trap records allow us to address potentially confounding relationships, for example involving age; iii) male and female Song Sparrows are identical in plumage traits and only show a small degree of sexual size dimorphism (Arcese *et al.* 2002); iv) they exhibit a low level of polygyny (Arcese 1989); v) despite a lack of sexually selected features, Song Sparrows are quite territorial and philopatric to territories suggesting a possible high degree of competition for them; Interestingly, the latter points suggest a situation where intra-sexual competition [for territories] is potentially uncoupled from other correlates of early male arrival (i.e. polygyny).

If Song Sparrows display reduced protandry, then I would expect males to exhibit weak evidence of intra-sexual competition for arrival time in the form of size- or condition-dependent arrival. Alternatively it should exist if they exhibit appreciable protandry.

I employed genetic techniques as a means to accurately sex Song Sparrows used in this study however the standard criteria used by banders to sex monomorphic species in the hand are reproductive characters (Pyle 1997). Sperm storage in the testes of males results in cloacal swellings, while in females vascularization of the abdominal skin aids in the transfer of heat to the eggs. Since all birds handled during this work were also subjected to normal banding procedures, I was afforded the opportunity to assess the accuracy of sexing on the basis of reproductive characters through comparison to genetic sexing, the results of which are presented in Appendix I. Although reproductive characters are considered unambiguous, error could occur and go unrealized in species where the sexes do not clearly differ in other ways. If they occur, sexing errors could be a function of human error in recognizing reproductive characters or biological variation in traits. Overlap in traits considered sexually dimorphic, such as size, plumage coloration and behaviour, is common. It is also recognized that females have the capability to produce cloacal protuberances morphologically similar to those in males through a physiological process other than sperm storage (Nakamura 1990).

CHAPTER 1

Migratory timing is predicted by plumage coloration in male Ruby-crowned Kinglets (*Regulus calendula*).

Introduction

Timing of spring migration is thought to be balanced by naturally selected costs and sexually selected benefits. Migration is costly in terms of the energetic demands, and arrival on the breeding grounds early in the season can result in mortality due to factors such as late winter storms (Brown and Brown 2000), an effect that is particularly problematic in insectivorous passerines. However, if early arrival confers a reproductive advantage, then those individuals able to pay such costs would benefit. The earliest arriving males on the breeding grounds can often acquire better quality territories, more mates and experience higher recruitment and multiple clutches (Møller 1994).

The selection pressure exerted on arrival time is thought to be a function of intra-sexual competition for breeding resources (Kokko 1999). Selection for early arrival causes the advancement of arrival date among males relative to females resulting in protandry. Thus protandry could reflect an outcome of competition for breeding resources, and should be correlated with other sexually dimorphic characters such as size and plumage brightness. Therefore in kinglets, a species with sexually dichromatic plumage and a large degree of protandry, males of higher phenotypic quality should arrive earliest.

Although it is well regarded that competition would result in condition-dependent arrival, often the evidence provided is indirect. Some authors have cited associations between arrival and plumage brightness but have used or included species with delayed

plumage maturation where age is a major factor also likely influencing arrival dates. Similarly Møller (1994) found that male Barn Swallows with larger retrices, a sexually selected trait shown to be condition-dependent in some cases, arrived on the breeding grounds earlier but this study lacked a direct determination of arrival time.

In this study, I investigate which aspect of phenotypic quality predicts arrival time of males and females of the Ruby-crowned Kinglet (*Regulus calendula*). The energetic demands of migration require sufficient stores of fuel and in birds these are most commonly lipids (Jenni and Jenni-Eiermann 1998). Fat is stored prior to departure and is refuelled at staging areas during migration (Sandberg and Moore 1996) therefore fat stores can influence time of arrival through departure date and rate of migration. Physically larger birds and those with higher fat loads can also more successfully withstand periods of fasting that accompany migration and harsh conditions early on the breeding grounds (Cristol *et al.* 1999). I consider body size and fat load and mass corrected for size as proximate predictors of arrival timing.

I also consider whether feather traits are associated with arrival times. Feather traits can be correlated with arrival time in spring indirectly through effects on feather growth at other times of the year. For example, coloration of secondary sexual traits and feather growth rate can reflect individual condition during the fall at the time of moult. Kinglets with faster feather growth rates as measured by growth bars and males with redder crests should arrive sooner but ultimately these measures should correlate with larger size and/or better condition. In other words, early arriving birds should be in better condition as assessed by proximate measures which in turn correlate with plumage characters. If plumage characters alone relate to arrival time, this could imply that aspects

of fall condition, potentially influencing fall migration, winter territory acquisition and over-wintering ability otherwise are potentially more important determinants of arrival time.

Materials and Methods

Study Site

Birds were collected at Ruthven Banding Station in Cayuga, Ontario Canada (N079° 52' 29.4'', W42° 58' 42.7''). Ruthven (see Fig. 1.1) is situated along the Grand River, which provides an inland migratory corridor for many species. Mist-net sites are chiefly situated in second growth habitat, primarily composed of *Cornus florida*, *Viburnum lentago*, *Rhus* spp. and *Crataegus* spp., separating a large tract of Carolinian forest and meadow. Mist nets were opened in mid-March and monitored regularly until June during the spring of 2003.

Study Species

Ruby-crowned Kinglets are insectivorous migrants over-wintering south of Ruthven and use the site as a staging area as they pass through on their migration to the breeding grounds in the north (see Ingold and Wallace 1994 for range map). The first birds typically arrive in mid-April and migration extends until mid-May, but individuals can arrive as early as late-march in favourable years and be caught until late-May in poor years. Ruby-crowned Kinglets show a large degree of protandry: Kissner *et al.* (2003) report that mean female arrival was 11.5 days later than males, one of the largest

Figure 1.1. Birds were trapped at Ruthven Park, Haldimand Bird Observatory located Southwest of Hamilton, Ontario. Ruthven is signified by an arrow at the bottom right corner of the figure.



differences in their sample of 30 sexually dimorphic species. Kinglets are easily sexed by the presence of a scarlet crest in males. The crest is fully concealable and revealed during agonistic and epigamic interactions (Ingold and Wallace 1994).

Measurements

Un-flattened wing chord was measured using a stopped metal ruler (± 0.5 mm). Mass (g) was taken using an Ohaus Scout SCD-010 balance (± 0.01 g). Residuals from body mass regressed on wing chord were used as a measure of body condition.

The amount of furcular fat and breast muscle was visually scored upon blowing the abdominal feather tracts aside. Fat was assessed as the amount stored in the depression at the base of the throat formed by the furculum: 0, no visible fat; 0.5, a slight amount visible on the floor of the depression; 1, floor of depression fully covered by fat; 2, level of fat roughly even with height of furculum; 3, fat extending beyond furculum. Muscle was scored similarly: 3, sternum indented due to concave muscle extending above height of sternum; 2, muscle originating from sternum not extending beyond height of sternum, muscle may slope off sternum forming a slight peak at sternum; 1, sternum prominent with muscle atrophied and slightly convex.

Colorimetric Analysis

Digital photographs were taken of the crest of each male and analyzed following Villafuerte and Negro (1998) for the degree of redness. Birds were removed to a dark room with a white fluorescent light as the only light source. To photograph the crest, the red crest was exposed by parting the body coverts while the bird was held under the light,

approximately 15cm away. All subjects were photographed from a distance of 30cm with the same shutter speed and f-stop using a Nikon CoolPix 950 digital camera. Images were opened in Adobe Photoshop and a section of the red crest was captured and imported to RGB Colour Software and analyzed. Only the red values were used in the analysis since we were interested in the degree of red in the crest. To further standardize across all pictures a sample of crest plumage was captured immediately to the right of where the red crest sample was taken and analyzed. I assumed that body plumage did not differ in the degree of red, and that any variation was due to colour bias between pictures. To standardize, I adjusted the mean red value of the body plumage for each male to be the same, then applied the same correction factor to the crest value. This mean corrected value was taken as the final score of plumage redness.

The length of the crest was measured using digital CE 300mm digital calipers ($\pm 0.01\text{mm}$) from the flattened crest by parting the crest feathers. The length of the crest (mm) was taken from the beginning of the red on the first anterior feather to the furthest fetch of red towards the back of the head.

Ptilochronology

The outer retrices (left and right r6) were collected before the birds were released. Feather growth bars were measured by pinning feathers down and marking off the bar that was closest to two-thirds the length of the feather measured from the culmen (Grubb 1989). From this point the bottom of the fourth distal (to culmen) and the fifth proximate bars were marked on the sheet of paper on which the feather was pinned. In most cases this represented nine alternations but where bars were difficult to see as few as five

alternations were used. This was repeated using a new piece of paper twice for each feather. In some cases feathers were soiled, damaged or naturally dull; feathers were omitted from the sample when bars could not be seen. For this reason a consistent feather (left or right) could not be used and so birds varied in the number of measurements they had (2-4). Because of the difficulties encountered sample sizes were low (males n=9; females n=6) and so results based on feather growth rates should be interpreted accordingly.

Statistical Analysis

All statistical procedures were carried out using R 2.0.0 (Ihaka and Gentleman 1996) statistical software except for regression models which were done using JMP 3.2.6 (SAS Institute 1999). Graphs were constructed using Sigmaplot 3.0.

I used Pearson's Correlation to examine relations between arrival date and measures of wing length (bird size). Residual Mass, as a measure of condition, is assumed to be a proximate predictor of arrival and so condition versus arrival was tested using ANCOVA corrected time of day. Since males and females differed in the response variable Julian Date, separate ANCOVA were done on each sex (with residual mass as the covariate). Time was categorized as two equal sampling periods: dawn to 9am, 9am to approximately 12:30pm; and was entered as a covariate along with the interaction term Residual Mass \times Time.

The relationship between Fat Score (a categorical variable) and Julian Date was investigated separately for each sex using Spearman's Rank Correlations.

As an additional measure of condition, I was interested in the relationship between feather Growth Rate and Julian Date. Variability in feather quality resulted in fewer measurements for some birds and an inconsistency in which (left or right) feathers were measured. Since a single consistent feather could not be used, and a single average value for each bird varies in the level of error depending on the number of measurements, I opted to bootstrap correlation coefficients. Growth Rates were randomized and correlated to Julian Date 500 times with an output of a single average correlation coefficient. The process was repeated for Crest Colour and Growth Rate.

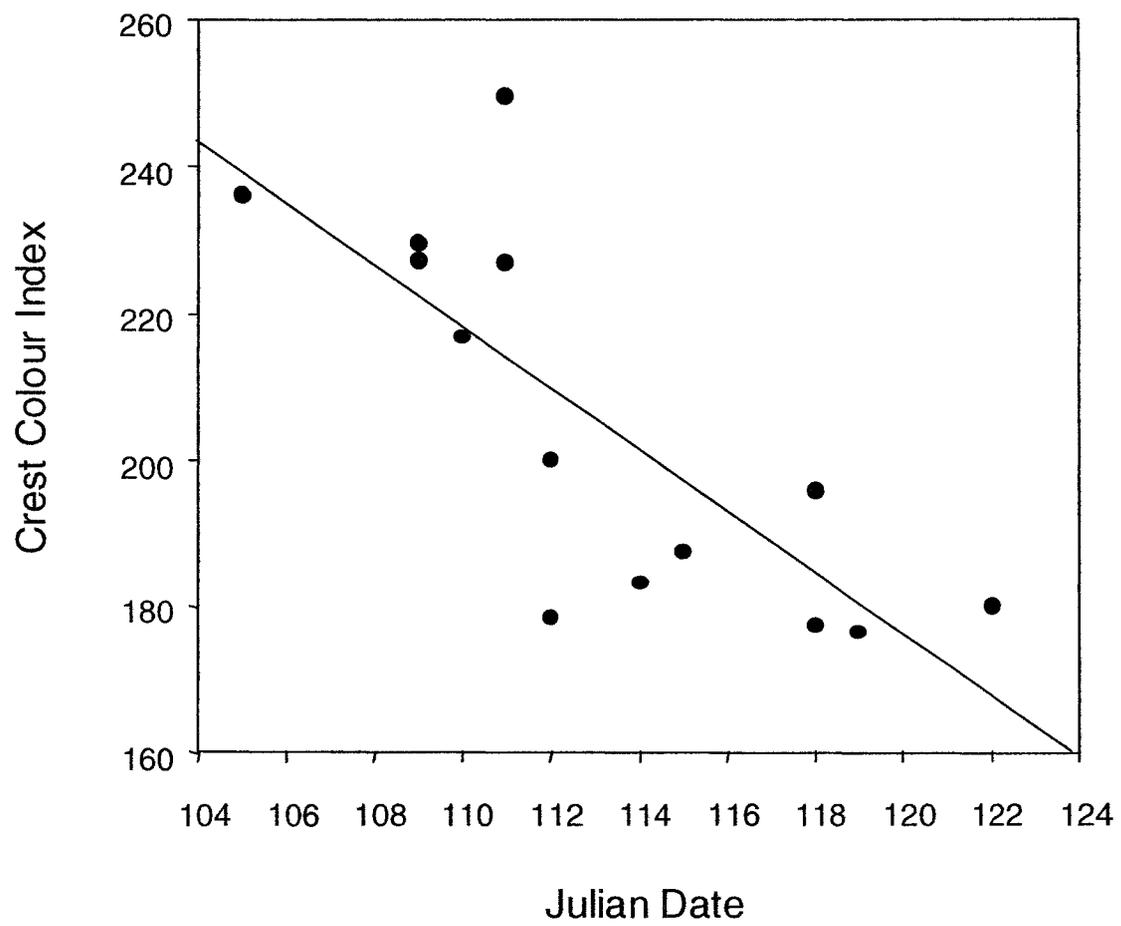
For the subset of males where both crest characteristics were available ($n=14$), Crest Colour and Crest Size were first tested for inter-correlations before being entered into a multiple regression with Julian Date as the response variable.

Results

Kinglet migration at our site began on April 16 and extended to May 15 in 2003. Male migration ranged from April 16 to May 5 ($n=21$); the first female was trapped on April 28 ($n=19$). The difference in mean arrival date for the sexes was approximately 12 days. Further analyses refer to the above sample sizes for males and females unless otherwise stated.

Male Size did not correlate with Julian Date ($r=-0.1293$, $P=0.58$) nor did Julian Date correlate with size in females ($r=-0.0507$, $P=0.84$). There was no relationship evident between Condition and Julian Date for males ($F_{1, 18}=0.02$, $P=0.89$; Time interaction $F_{1, 18}=0.005$, $P=0.94$) or females ($F_{1, 17}=0.63$, $P=0.44$; interaction $F_{1, 17}=0.14$, $P=0.71$). Likewise, male ($\rho=0.27$, $P=0.24$) and female ($\rho=-0.20$, $P=0.41$) fat score did

Figure 1.2. Crest Colour relative to arrival date in male Ruby-crowned Kinglets (P=0.001). Crest Colour value was determined by colorimetric analysis of digital photographs, standardized across individuals using values measured from body plumage (see text). High values indicate more red in the crest plumage. Julian Day 1 equals January 1, 2003.



not relate to arrival date. Muscle proved to be invariable and was excluded from the analysis.

Growth rate showed a high degree of variability between feathers from the same bird and in some cases measurements from the same feather. Given this low repeatability and low sample size (males: $n=9$; females: $n=6$) it is not surprising it did not correlate with arrival date in males ($P=0.73$) nor in females ($P=0.92$). Retrices and crest plumage are moulted in approximately at the same time, at the time of the pre-basic moult in late summer/early fall. A brighter crest should be associated with faster growth rates if both characters are the product of superior condition of the owner. I found no evidence to suggest that the traits were correlated.

Arrival date was highly related to Crest Colour ($F_{1,12}=18.53$, $P=0.001$; Fig. 1.2); males with redder crests arrived earlier in the migratory period. However, Crest Size was not significantly related to arrival date ($F_{1,12}=1.06$, $P=0.3254$).

Discussion

Consistent with published accounts for Ruby-crowned Kinglets, mean male arrival date preceded females by approximately 12 days. In comparison to other species this is a relatively large degree of protandry; for instance in wood warblers, males precede females by an average of 4 days (Francis and Cooke 1986). These results are in accordance with the hypothesis that the competing sex faces competition for breeding resources that selects for early arrival. Intra-sexual competition is thought to advance the arrival date in the competing sex relative to the other which could be manifest as condition-dependent arrival (Kokko 1999). The only trait I measured that correlated with

arrival date was the redness of the crest in males. Males with redder crest plumage arrived earlier than those with duller crests, potentially suggesting a condition-dependent link to migration timing in kinglets.

The condition dependence of sexually dimorphic plumage characters has garnered considerable attention recently and males with exaggerated traits have been consistently shown to be in superior condition. Specifically carotenoid-based plumage has been shown to vary with nutritional and immunological condition (Brawner *et al.* 2000, Hill and Montgomerie 1994). Fewer studies have revealed in species bearing sexually selected traits that males with elaborated traits also arrive and initiate breeding earlier (Møller 1994, McGraw *et al.* 2001).

As migration is energetically costly (Alerstam 1990) it is interesting that my proximate measures of condition (i.e. fat score and residual mass) did not relate to arrival time. That fat stores can mediate the effects of energetically costly migration, affecting an individual's decision to leave the wintering grounds, the rate at which it travels and ultimately its arrival on the breeding grounds, is clear. Fat is important for migrating birds as it is used to meet the energetic demands of migration, to survive harsh conditions early on the breeding grounds and in addition, condition at breeding onset can predict reproductive success (Sandberg and Moore 1996). Furthermore arrival date has been shown to be condition-dependent in this sense in other species (Gustafsson *et al.* 1994, Piersma and Jukema 1993; see also Chapter 2) and fat levels are known to correlate with other components of condition in a closely allied species, the Goldcrest (i.e. hematocrit; Merila and Svensson 1995). The lack of a relationship between arrival date and condition (fat score or residual mass) may suggest that an individual's ability to migrate, with

arrival time being a function of departure date and rate of migration, is not clearly dependent on energetic condition in Ruby-crowned Kinglets. A relationship between fat load and arrival at Ruthven could have been obscured if the difference in fat load at time of arrival to and departure from the staging area (Ruthven) were larger than the general variation of individual quality predicting timing of migration. If individuals who recently arrived at Ruthven are in poor condition compared to those that have fattened up and are leaving, the status that predicted their arrival date could be swamped by a relatively large difference between arrival and departure condition at the staging area. With this said, similar error would be inherent of all studies, yet I clearly observed condition-dependent arrival in male Song Sparrows in Chapter 2.

Whether condition is directly expressed in the redness of kinglet crests has yet to be shown. Due to the popularity of the Immunocompetence Handicap Hypothesis (see Folstad and Karter 1992), the majority of studies focus on the link between plumage coloration and immunological traits rather than energetic aspects of condition. Crest colour predicting arrival date via a link to immunological condition could be the case for kinglets. Gustafsson *et al.* (1994) suggested that the energetic debts in migrating birds could be offset through allocation of energy from other processes, particularly the immune system. However given the sizeable input of energetic condition to migratory ability (see Jenni and Jenni-Eiermann 1998), I question whether proximate variability in immunological condition can be solely responsible for arrival patterns.

An alternative to this idea- that kinglet crests reveal individual condition and that this is functionally linked to arrival time - is that the crest is used during agonistic interactions for resources and that the winners of these contests may be better able to

migrate early and/or efficiently. The red crest of kinglets is completely concealable behind body coverts, so much so that males can easily be mistaken for females if the crown feathers are not parted. It is often suggested that the ability to conceal a trait is important in preventing costly agonistic interactions: a concealed trait signifies subordination while an exposed trait provokes aggression. Indeed, kinglet crests are fully exposed – raising the crest from front to back and extending red feathers laterally – in a variety of agonistic displays, yet are not revealed to this extent to potential mates during mating displays (Ingold and Wallace 1994). This may suggest that crests play a more important role in interactions with competitors than to potential mates.

I was unable to find evidence in the literature of condition-dependence of a concealable trait, although tests of these characters are uncommon. Studies of Red-winged Blackbirds have not been able to reveal correlations among obvious aspects of condition (i.e. parasite load, mass corrected for body size) and features of the red epaulets of males (Weatherhead 1990, Weatherhead *et al.* 1993). However epaulet size, but not colour, has been shown to relate to dominance status and nest defence, traits that improve reproductive success (Hansen and Rohwer 1986, Searcy 1979) which has led to the idea that the size of these concealable plumage traits is more important than colour. In Kinglets, crest size did not correlate with arrival time whereas crest colour did suggesting a disconnect in the expression of the trait since birds with brighter crests did not also have larger crests.

Feather growth bars were used as a measure of condition at the time of moult. A positive relationship between growth rate and colour would imply that crest colour is a result of nutritional condition during moult. The two were not correlated but I question

the validity of the results based on low repeatability and sample size. Although the technique is often used with success its reliability has been called into question (e.g. Kern and Cowie 2002, Murphy and King 1991).

Since we do not know the status of kinglet crests I am unable to differentiate whether arrival might be due to condition or some other aspect of competitive ability due to signal expression. In either case though, it is likely that the relationship between arrival and crest colour is not direct. Kinglets undergo a single pre-basic moult in the late summer when the crest plumage is moulted. Therefore the expression of the trait as measured in the spring is likely a mere correlation with the fall conditions under which it was moulted. Individuals displaying good signals may be inherently in good condition allowing them to migrate early. Alternatively, fall signal status, whether condition-dependent or not, may allow an individual to obtain a favourable territory which translates into early arrival (Chandler and Mulvihill 1990). Consistent with this idea, male Ruby-crowned Kinglets are territorial on the wintering grounds and males over-winter farther north (Ingold and Wallace 1994).

In summary, I found no evidence of selection for female arrival but male arrival was predicted by plumage coloration of the crest. Contrary to expectations, my proximate measures of condition did not predict arrival, which is interesting given that migration and occupying the breeding grounds early in the season is quite costly energetically. The finding that the secondary sexual character of males relates to arrival is consistent with other studies, and the idea that arrival time is also sexually selected. However, the aspect(s) of the trait that is advantageous remain unknown. Concealable traits are known to be used primarily in agonistic battles where trait quality can predict the outcome. But

whereas size is regarded as important in these instances colour is not. Alternatively the colour of plumage characters has been shown to relate to condition of the owner at the time of moult, and is most often the aspect of plumage traits preferred by females. In kinglets there is conflicting evidence as to whether individuals with bright crests are benefited through a link to condition-dependence or directly through agonistic interactions. It is also unknown whether arrival is mediated proximately through an aspect of condition unmeasured or whether it is predicted via an association with overwintering ability associated with signal quality or condition at the time of moult.

CHAPTER 2

Concomitant selection for early arrival of females may reduce the degree of protandry in the monogamous Song Sparrows (*Melospiza melodia*).

Introduction

Breeding onset and competition for breeding resources are important factors shaping migration ecology in birds (Kokko 1999). Individuals that arrive early often experience improved reproductive success by acquiring more mates, better territories, have a higher likelihood of multiple clutches and higher recruitment rate of early fledged offspring (Møller 1994). Differential competition for breeding resources within a sex is thought to be the mechanism which advances the arrival date of one sex relative to the other (Kokko 1999). The most common manifestation of this is protandry, where males precede females to the breeding grounds.

The extent of protandry has been shown to correlate across a number of species with the level of sexual size dimorphism (Kissner *et al.* 2003) and dichromatism (Rubolini *et al.* 2004), which in turn are often related to mating system (Dunn *et al.* 2001, Møller and Birkhead 1994). Since species that show the highest degree of sexual size dimorphism, dichromatism and polygyny also have the greatest difference in arrival dates between the sexes, protandry is thought to vary with degree of sexual selection experienced by males. If this holds, the prediction follows that monomorphic passerines should display a lack of early male arrival. Given the difficulty in sexing monomorphic species, they are often omitted from multi-species comparative studies (e.g. Kissner *et al.*

2003, Rubolini *et al.* 2004) and so little empirical data are available for arrival dates of these species.

Alternatively, monomorphic species may be subject to wholly different selective forces in terms of relative arrival dates. For instance, in species where males and females closely resemble each other, delayed female arrival may help them avoid the costs of male aggression early in the season; a period when males are aggressively establishing territories. Postponing their arrival until males have defined their territories may be an adaptive behaviour to avoid injury and stress in females of largely monomorphic species.

Given that sexual selection appears to be related to migration timing, studies often focus on males, however breeding onset is also an important determinant of female reproductive success (Price *et al.* 1988). We include both sexes in our analysis of selection for arrival timing in Song Sparrows (*Melospiza m. melodia*).

Study Species

In the hand Song Sparrows (*Melospiza m. melodia*) cannot be accurately sexed based on size or plumage as they are only mildly size dimorphic and have identical plumage (Pyle 1997). Song sparrows demonstrate a low level of polygyny with only 9.5% of matings occurring in polygynous groups (Arcese 1989). This level of polygyny is low in comparison to species classified as polygynous (as high as 100% in red-winged blackbirds; Yasukawa and Searcy 1995) as well as many considered socially monogamous (up to 30% in the American Redstart; Omland and Sherry 1995). The degree to which Song Sparrows undergo extra-pair copulations has not been genetically determined.

Although there seems to be little evidence of mate selection based on physical traits, territory quality is an important determinant of reproductive success and both sexes are territorial, actively excluding members of the same sex from their territory (Arcese *et al.* 2002) suggesting there is intra-specific competition for resources.

Materials and Methods

Study Site

Song Sparrows (*Melospiza m. melodia*) were trapped as they arrived on the breeding grounds at Ruthven Park, Cayuga, Ontario, Canada (N079° 52' 29.4'', W42° 58' 42.7''). Ruthven is located adjacent a major river system with a wet floodplain that supports a healthy breeding population of Song Sparrows. A large Carolinian slough forest and agricultural lands bordering the banding site also provide suitable breeding habitat.

In southern Ontario, Song Sparrows are considered migratory with only a very few individuals over-wintering (see Arcese *et al.* 2002 for range map). The 2003 Hamilton Naturalists' Club Christmas Bird Count recorded only 23 Song Sparrows in an area encompassing approximately 120,000ha in which Ruthven is included (Hamilton Naturalists' Club 2004). Harris and Lemon (1976) report breeding densities of 3.3 pairs/ha in a similar habitat and latitude, and it is possible to see approximately 40 birds in a single hectare of floodplain at Ruthven during the breeding season. Consequently, the number of over-wintering birds is insignificant and should not confound estimates in arrival dates of the sexes.

Sampling Period

The main study occurred during the spring of 2003. Mist-netting began on March 15 and continued in a rigorous manner until the end of June. To improve my sample size in comparisons between the sexes, a number of birds were also sampled during the spring of 2004.

Field Protocol

Trapping Song Sparrows followed guidelines of the Canadian Wildlife Service (1977). Mist-nets were opened prior to sunrise and monitored for approximately 6 hours during suitable weather. Birds were banded with a unique metal leg band and processed.

Mass (g) was measured using an Ohaus Scout SCD-010 balance (± 0.01 g) and size was measured as un-flattened wing chord, taken to the nearest millimetre using a stopped metal ruler (± 0.5 mm). Although observed wing length can result from other relationships (e.g. Harper 1999), it is often used as a measure of body size in other studies (e.g. Kissner *et al.* 2003) and is currently accepted as the measure of body size by the Canadian Wildlife Service and the United States Fish and Wildlife Service (Canadian Wildlife Service 1977). Additionally, in Ruby-crowned Kinglets keel length and wing chord are correlated ($r^2=0.24$, $P=0.01$; unpubl. data).

Fat was scored on a 3 point scale. The abdominal feather tracts were parted by blowing up the breast and the amount of furcular fat was categorized as: 0, no fat stored in depression created by the furculum and the base of the throat; 0.5, trace amount in the floor of the depression; 1, floor of depression fully covered by visible fat; 2, depression

filled approximately level with furculum; 3, fat extending beyond furculum. Condition was scored relatively as body mass corrected for size. Residual body mass was determined separately for each sex from a regression of mass on wing chord (mm), as was done in Chapter 2 for Ruby-crowned Kinglets. Before being released feather samples were collected for genetic sexing following the procedures outlined in Appendix I.

I relied on re-capturing a certain portion of the sample during the spring and early summer of 2003, as well as re-capture records from years prior to the field season, in order to determine age, track changes in condition over the breeding season and to estimate which birds were local breeders.

Age was established for the subset of birds that had been previously trapped, and were designated as Second Year (SY: $n=11$) or After Second Year (ASY: $n=15$). To this end, I could determine age in approximately half the sample in one of two ways. A small portion of the 2003 sample had been trapped in a previous year and were therefore known to be ASY when they were trapped in 2003. A large portion of the known-age birds were originally trapped in the fall of 2002 and were aged as Hatch Year and After Hatch Year (equivalent to SY and ASY in the spring of 2003) based on skull morphology.

Ossification of a second layer of cranial bone occurs gradually after hatching. Crest feathers were parted to observe a single pink layer of bone surrounded by white, fully ossified skull (double layer) in Hatch Years or as a completely white skull in After Hatch Years. Since the progression of the ossification follows predictable patterns, skull morphology is an effective aging method (Pyle 1997).

Birds were classified as Local Breeders if they were re-captured between the period of June 1 and August 15, especially if they possessed reproductive characters.

Statistical Analysis

Data were analyzed using R 2.0.0 statistical software (Ihaka and Gentleman 1996) except for ANCOVA models which were done with JMP 3.2.6 (SAS Institute 1999). Graphs were designed in Sigmaplot 3.0. Wing chord and mass were compared between the sexes using t-tests to address the degree of size dimorphism, with the sample being composed of genetically sexed birds from both sampling years (2003 and 2004). Median arrival date was compared between the sexes using a Wilcoxon Rank Sum Test for the largest sampling year only (2003) with 14 males and 29 females recorded; due to a small sample size in 2004 (7 males and 15 females), tests of sex-biases in arrivals were not done.

For the reason outlined in Chapter 1, Pearson's Correlation was used to test Julian Date versus Wing Chord for both sexes. This relationship could be confounded if smaller, local breeding sparrows arrive at a different time than larger birds continuing north. Among western subspecies, there appears to be a latitudinal gradient in size (Arcese *et al.* 2002) with the largest individuals in the north consistent with Bergmann's Rule. Birds were considered local breeders if they were trapped between May and August. All birds not trapped during this time period were assumed to be non-local breeders.

Fat score was examined in relation to arrival date using Spearman's Rank Correlation. ANCOVA was used to correct for sex and time effects in the relationship between arrival date and condition. Julian Date was entered as the response variable with Residual Mass as the effect. Residual Mass \times Time and Residual Mass \times Sex were

entered as interaction terms. Time of day was classified as: dawn to 9am, and 9am to 1pm.

A relationship between arrival and residual mass, whether negative or positive, could be a function of, or complicated by, a decline or improvement in condition over the season (Christe *et al.* 2002). To address this, I used a paired t-test to assess whether individual condition (residual mass) changed in a predictable manner between arrival date (first trapped) and two and four weeks later (depending on re-trap date) in a subset of individuals re-trapped during the spring of 2003. The analysis using residual mass was done by entering all individual values of wing chord (during migratory period and at re-trap date) into a regression. The residuals values were removed from the regression and paired for analysis.

Results

Sample and Genetic Sexing Results

In 2003, feather samples were taken from 52 individuals from which 43 were successfully sexed. Of these, 29 were females and 14 males (See Appendix I). In 2004, only 22 birds were sampled at the beginning of the migratory period. This sample had a similar proportion of females versus males as did earlier samples (i.e. 15 females and 7 males).

The actual migratory period for 2003 was estimated by removing outliers from box plots of arrival dates for each sex. In doing so, the range of arrival dates for males was truncated to Julian Dates 84-119 (n=12), and 84-116 (n=27) in females.

Relationships between individual metrics and arrival dates were explored using only these individuals.

Differential Arrival

In 2003 median arrival date was Julian day 101.5 in males and 100 in females, when taken for all individuals trapped over the sampling period ($P=0.92$). Using the sample retained for intra-sexual analyses, the median shifted to 100 in males and 99 for females. The first birds were captured on day 84 when a single male and female were trapped. Peak captures occurred approximately 2 weeks later and continued throughout the spring.

Sexual Size Dimorphism

Male Song Sparrows ($n=19$) are larger than females ($n=38$) as measured by mass ($P=0.038$) and wing chord ($P=0.004$). Despite the significance of this finding there was substantial overlap in both measures. Mean male mass (21.2g) was larger than mean female mass (20.2) but male weights were less variable (min 19.1g, max 23.7g) than that of females (min 16.8g, max 26.9) such that both the lightest and heaviest birds were female. Similarly mean wing chord was larger for males (65.6mm) than females (63.9mm). Females ranged from 60-67mm and overlapped with much of the male range (62-69mm).

Intra-sexual Arrival

As noted above, it can be difficult to determine when the migratory period has been completed (new captures do not necessarily mean new arrivals). I was interested in the

status of individuals during the migratory period and not over time after arrival for Song Sparrows breeding locally. I approximated the migratory period by removing outliers from the latter tail of the distribution observed using box-plots of Julian date, separately for each sex.

Regressions and correlations were carried out on only those females (n=27) and males (n=12) genetically sexed during the spring of 2003.

There was no correlation between size, as measured by wing chord (mm), and arrival date in males ($r=0.08$, $P=0.81$). However in females larger individuals arrived significantly earlier than smaller females (Fig. 2.1; $r=-0.45$, $P=0.02$). This relationship would be confounded if younger smaller birds arrived later than larger older birds, a pattern documented in other species. I found that SY birds (n=8) did not differ from ASY birds (n=9) in size ($P=0.98$) or arrival date ($P=0.60$). The results of Davis and Arcese (1999) confirm that these age groups do not show differences in migratory tendencies. Similarly the pattern in females could be produced if larger individuals were headed for breeding grounds further north and passed earlier relative to smaller, locally breeding sparrows. A separate correlation examining size and arrival date and using only local birds (n=16) was highly significant (Fig. 2.1.; $r=-0.49$, $P=0.005$).

Spearman Rank Correlations revealed that early arriving males carried significantly larger fat loads (Fig. 2.2.; $\rho=-0.807$, $P=0.003$) than those arriving later in the migratory period. Female arrival date was not dependent on fat score ($\rho=0.041$, $P=0.83$).

The relationship between arrival time and body condition was negative and weak ($F_{1, 33}=1.94$, $P=0.17$). There was no interaction between Time and Condition

Figure 2.1. Females that arrived on the breeding grounds earlier were larger than those trapped later ($P=0.02$). A similar trend existed among only those females known to be local breeders ($P=0.005$) and so breeding locality was not a confounding factor. Additionally there was no difference in arrival dates of SY and ASY birds (see text). Julian Day 1 equals January 1, 2003.

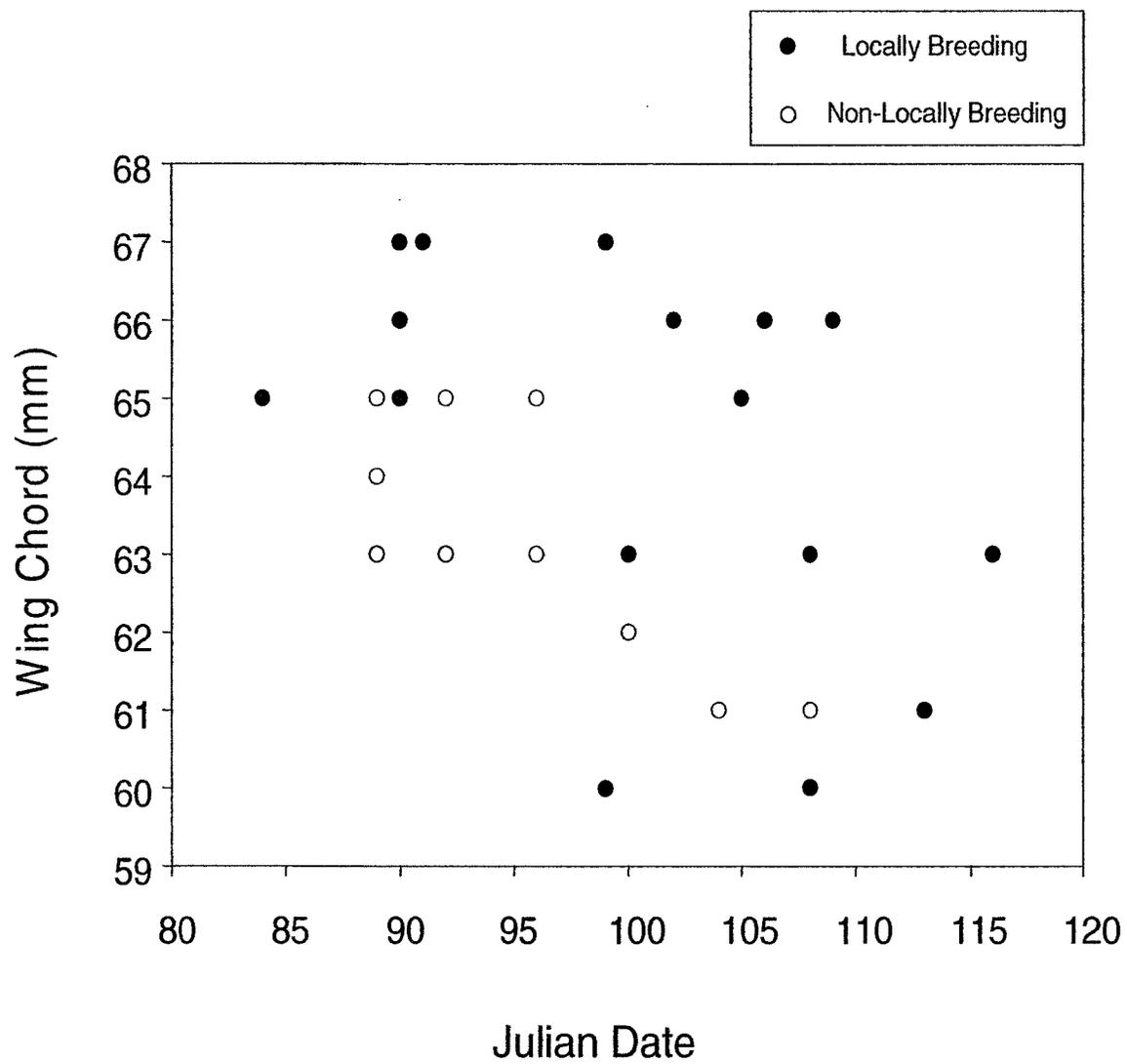
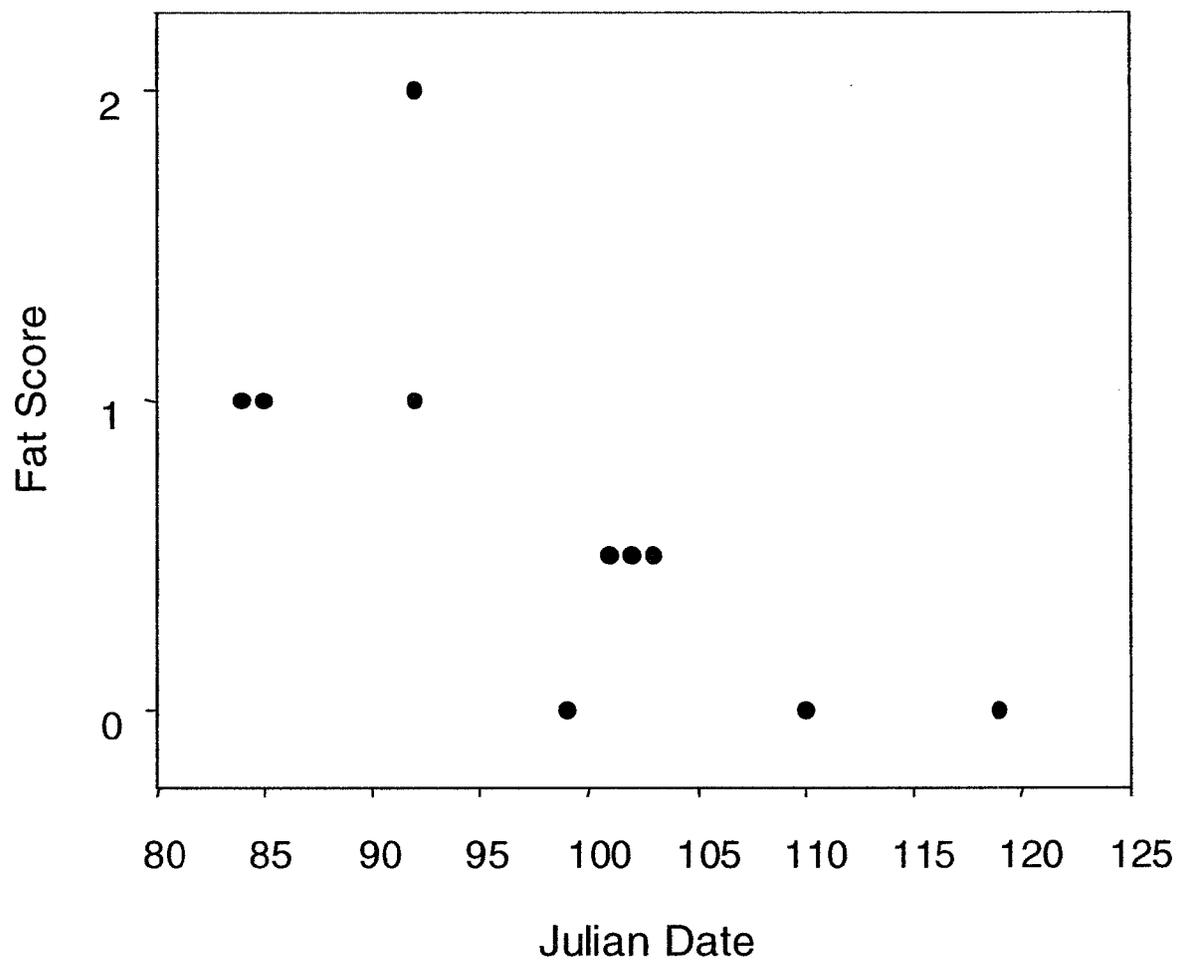


Figure 2.2. Fat score at time of capture in male Song Sparrows ($P=0.003$). Males in better energetic condition were trapped earlier. Fat score was determined on a relative scale (see text). Julian Day 1 equals January 1, 2003.



($F_{1,33}=0.21$, $P=0.65$) but there was a near significant interaction between Sex and Condition ($F_{1,33}=3.53$, $P=0.07$). Males with higher Residual Mass had a tendency to arrive earlier while a similar relationship did not exist for females.

Changes in condition could be a function of physiological preparations for breeding, such that declines in condition covary relative to the onset of the breeding season rather than arrival date. A paired t-test showed that condition did not change in a predictable manner in males ($n=8$) between time of first capture and 2-6 weeks later ($P=0.408$).

Discussion

Unlike sexually-dichromatic species with early male arrival, song sparrows show little evidence of protandry. In fact, I found male and female median arrival dates to be virtually the same. I have no reason to suspect that early arriving males were less susceptible to being caught in nets, which would have shifted the median arrival date in males later. Passive netting, whereby the assumption is made that first capture date is a useful measure of arrival date, has been used with success in other studies (e.g. Lozano *et al.* 1996), and this technique is capable of detecting sex differences in arrival date in other species at our field site (Ruby-crowned Kinglets; see Chapter 1).

Nice (1937) reported male Song Sparrows arrived earlier than females. This population is partially migratory and shows a larger degree of sexual size dimorphism so may reflect a true difference between the populations, but could also be explained by a difference in sampling methods. Observational data on arrival dates may be biased if one sex is more obvious. This could be a problem in studies of sparrows where males call

from exposed perches while females remain relatively concealed (where the sexes are otherwise similar). Females remaining hidden at this time may be a behavioural modification to avoid male sexual and territorial aggression, as females do not appear to avoid male aggression by avoiding the breeding grounds early in the season.

The population of Song Sparrows at Ruthven displayed limited sexual size dimorphism. Mean size and mass were larger in males than females but the degree of overlap was extensive. Wing chord measurements for all birds genetically sexed ranged from 60-69mm with males and females overlapping from 62-67mm. Size measurements showed no evidence of a bimodal distribution; 81% of the individuals in the sample fell within the range of overlap. This degree of overlap is consistent with published accounts of *Melospiza m. melodia* (Pyle 1997). Arcese and Smith (1985) on the other hand were able to accurately sex over 90% of their Song Sparrows (a west coast, non-migratory population) by using discriminant function analysis on size measurements. Similarly, Nice (1937) reported that males were clearly larger than females such that they could be sexed using wing chord alone. Her sample appeared to be clearly bimodal with only a small number of individuals falling within the range of overlap in size of the sexes (range of overlap 62-66mm (50% of size range); mean male wing chord 66.3mm; mean female wing chord 62.1mm). Not surprising males at Ruthven were significantly larger than females in terms of body mass although the heaviest and lightest birds were both female and thus there is complete overlap of the sexes in potential weights.

I demonstrated that female Song Sparrows display size-dependent arrival irrespective of age, suggesting that larger individuals are better able to arrive early. Chandler and Mulvihill (1990) found that within age groups larger male juncos passed

earlier and posited that this could be due to intra-sexual selection for early spring arrival. In males of a variety of species, larger individuals arrive earlier and acquire better territories (Bédard and LaPointe 1984, Hasselquist 1998). In general, larger individuals have been shown to have a competitive advantage for resources through social dominance (Richner 1989) and are better able to cope with adverse weather conditions (Brown and Brown 1998). Their large size could also enable them to over winter farther north and/or migrate earlier. Furthermore birds in better condition at the time of moult grow relatively larger wings (Thompson *et al.* 1997).

My finding that males in better condition (with higher fat load and residual mass) arrive earlier than males in poor condition is not surprising. Males of many sexually dimorphic species demonstrate a similar trend with respect to body condition, immune condition or condition-dependent plumage (e.g. Gustafsson *et al.* 1994, Hasselquist 1998, Møller 1994, Ninni *et al.* 2004, Phalen *et al.* 1995, Piersma and Jukema 1993). Males benefit from early arrival in terms of higher reproductive success: early arriving males gain superior territories (Aebischer *et al.* 1996, Bensch and Hasselquist 1991, Currie *et al.* 2000), have a higher frequency of matings (Hasselquist 1998, Lozano *et al.* 1996, Møller 1994) and produce more clutches with higher recruitment (Møller 1994). Migration is energetically costly (Jenni and Jenni-Eiermann 1998, Piersma and Jukema 1990), with exaggerated costs early in the season when conditions are harsher on the migratory route and breeding grounds (Brown and Brown 2000). Thus migration onset, rate and ultimately date of arrival should depend upon the condition or size of the individual. The resulting intra-sexual selection for early arrival due to competition for

breeding resources appears to be manifested as condition dependent arrival (see Kokko 1999).

The fact that Song Sparrows display reduced early male arrival, limited sexual size dimorphism, no dichromatism and a low frequency of polygyny is consistent with predictions relating to sexual dimorphisms and early male arrival (size dimorphism, Dunn *et al.* 2001; dichromatism, Rubolini *et al.* 2004). Current explanations of EMA focus on the benefits to male arrival whereby the benefits to early arrival are higher in polygynous species because the difference between reproductively successful and unsuccessful birds is exaggerated. Intra-sexual competition for breeding resources consequently advances the arrival dates of the competing sex relative to the other causing protandry (Kokko 1999), and protandry to a higher degree would be expected where male-male competition is exaggerated, such as in polygynous and otherwise strongly sexually dimorphic species (see results of Chapter 1 and Chapter 2). Given the universality of the benefits to early arrival though: more clutches, better territories, better mates, higher recruitment; one would expect these to be sufficient forces to select for noticeable protandry among most, if not all, species of passerines. Given that male song sparrows display condition-dependent arrival to the breeding grounds (evidence of intra-sexual competition for arrival), aggressively compete for breeding resources (i.e. territories) and presumably are subject to the universal benefits of early arrival, why has this not resulted in an advancement of male arrival dates relative to females? Reduced benefits to early arrival in males given reduced polygyny is certainly one explanation however I suggest that simultaneous selection among females may also advance relative female arrival dates and reduce the degree of perceived protandry in this population of Song Sparrows.

Amongst monogamous females it is evident that there is also strong female-female competition for breeding resources. Female song sparrows are aggressive towards other females, but not usually towards males (Arcese 1989). Males of otherwise monogamous species will take multiple mates if the opportunity presents (Veiga 1992), but in Song Sparrows, and indeed among monogamous females in a variety of species, female aggression is important in maintaining their monogamous status (Arcese 1989, Liker and Székely 1997, Slagsvold 1993, Veiga 1992). Actively excluding other females from their territory and preventing polygynous matings improves their reproductive success such that monogamous females gain full access to the resources of their mate and his territory (Hannon 1984).

In support of this, female Song Sparrows exhibit a high degree of site fidelity (Nice 1937). Prior breeding success at a site is an important determinant of the level of individual philopatry in other species. Females having high breeding success the previous season exhibit strong site fidelity and attempt to re-acquire high quality territories while those with low prior breeding success attempt to relocate to better territories (Blancher and Robertson 1985, Hoover 2003, Johannesen *et al.* 2002). Strong site fidelity suggests that territory quality is important for female Song Sparrows and may be a source of intra-sexual competition. Early arrival may enhance the ability of females to secure the best territories in a system where sharing of resources is limited.

If Song Sparrows represent an extreme case where there is little EMA and potentially strong female-female competition, species with a large degree EMA should lack selection for female arrival. Chandler and Mulvihill (1990) found evidence for selection on wing morphology in male but not in female in the protandrous Dark-eyed

Junco. Similarly, male Ruby-crowned Kinglets migrate substantially earlier than females (mean difference between sexes is approximately 12 days; Chapter 1) and male arrival is predicted by plumage coloration (see Chapter 1) but there was no evidence of selection for early arrival in females. Evidence of strong intra-sexual competition for arrival in males and a lack of selection in females causing a large difference in arrival dates is consistent with the idea that independent selection on both sexes accounts for the degree of protandry.

Females of philopatric, monogamous species may exhibit especially strong intra-sexual competition resulting in an advancement of arrival date for the following reasons:

- i) Where females actively exclude other females from their territory and prevent polygynous matings, late arriving females increase the risk of being unmated if there is a sex bias or are limited territories. There is evidence to suggest that there can be a reserve of unmated females in monogamous species (Nice 1937) whereas in polygynous species all females are usually mated.
- ii) Unsuccessful breeders looking to acquire a better territory/mate to improve reproductive success must precede other females to do so. It is evident from studies of males that early arriving individuals get access to better territories (Aebischer *et al.* 1996, Brooke 1979, Lozano *et al.* 1996) and territories are usually more successfully defended by the first bird to arrive at it (Orians 1980). With previously unsuccessful females and new recruits to the breeding population attempting to acquire [better] territories, females must return early to secure their breeding resources. Arriving late to the breeding grounds to find another female already occupying your territory is much less costly in polygynous species since territory occupation by one or more females does not exclude a late arriving female from those resources. Therefore selection for

arrival date among females of polygynous females must be reduced compared to monogamous females.

As indicated, male Song Sparrows display condition-dependent arrival, circumstances thought to evidence competition for breeding resources and necessary for the advancement of relative male arrival date, but we found little evidence of protandry. Although reduced, I suggest that intra-sexual selection for male arrival date likely does exist however, but that it is obscured by simultaneous selection for early female arrival date as evidenced by size-dependent arrival. Costs to late arrival for females are predicted to be greater in monogamous species resulting in intra-sexual selection for early arrival and the relative advancement of arrival dates. Thus the pattern of limited protandry in monogamous species may be a result of reduced selection in males and increased selection in females.

SUMMARY AND CONCLUSIONS

Competition for breeding resources is often high in males and males frequently bear traits attesting to competition and female choice, such as sexually dimorphic plumage.

Therefore studies interested in explaining processes and patterns of sexual selection focus their attention where evidence of sexual selection is obvious. Eliminating females and sexually monomorphic species may limit the applicability of these studies in the larger sense. In this thesis I was concerned with characterizing intra-sexual patterns in both sexes with respect to arrival in a species showing early male arrival. Secondly I was interested in whether a sexually monomorphic species displayed early male arrival, and if this correlated with the presence or absence of selection for intra-sexual arrival.

Ruby-crowned Kinglets, a species with a large degree of protandry, exhibit plumage-dependent arrival. Males with redder crests arrived earlier than those with duller crests. Expression in terms of size of concealable traits, such as the crests in kinglets, has been shown to lead to a competitive advantage in agonistic battles. However, males with larger crests did not arrive earlier and crest size was not related to crest colour contrary to predictions. Plumage coloration, and specifically carotenoid-based plumage, has been shown to be condition-dependent whereas with concealable traits benefits typically tend to be independent of colour. The evidence is contradictory and so more study is needed into what determines crest colour in kinglets and in what way the benefits are reaped from having such a trait.

I did not find evidence for a direct link between condition or size and arrival time or crest colour. Arrival may correlate with an unmeasured aspect of condition. Alternatively, spring arrival time may be related to aspects of condition or migratory

tendencies in the autumn. Individuals with redder crests may have an advantage in acquiring good winter territories due to superior condition at the time of moult, or by possessing a signal which benefits them in agonistic competition for these resources.

Correlations between sexual size dimorphism, sexual dichromatism and mating system predict that monogamous, monomorphic species lack the level of intra-sexual competition required to create early male arrival. In some monomorphic species though, males are reported to arrive significantly earlier than females, but there is a general lack of studies that quantify this empirically using standard sampling methods. I found that Song Sparrows clearly do not display the level of protandry that has been suggested to occur in some monomorphic species, and that is also observed in sexually dimorphic species. Song Sparrows are highly territorial though, so it would be expected that males would benefit from arriving as early as possible on the breeding grounds. Male Song Sparrows that arrived early had significantly heavier fat loads than those arriving later. Larger females arrived earlier than smaller females. I suggest that competition for breeding resources in females of highly monogamous, territorial species such as Song Sparrows can create situations where selection for arrival exists in both sexes effectively eliminating protandry.

In the course of genetically sexing Song Sparrows, I realized errors had been occurring in the assignment of sex based on reproductive characters. A relatively small portion of females were being mistaken for males. These characters are used across North America to sex monomorphic passerines so there is potential for banding records to contain errors. Since experienced banders were responsible for making these mistakes, I do not believe that they are solely due to human error. Overlap in traits considered

sexually dimorphic is common and so there overlap might exist in cloacal morphology in breeding males and females. I found that only larger females were being mistaken for males when size is not used as a sexing criterion. This implies that the sample of mistaken females is not random.

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APPENDIX I

Errors in sexing passerines using reproductive characters: a case study with

Melospiza m. melodia.

Introduction

In many species of passerines, distinguishing between the sexes can be exceedingly difficult. When there is a large degree of overlap in body size and coloration of males and females, birds may only be sexed using reproductive characters present during the breeding season. At the onset of the breeding season, the male cloaca of many species of passerines enlarges into a recognizable cloacal protuberance (Pyle 1997). The function of the protuberance is to facilitate the storage of sperm and to aid in copulation. Prior to laying, females develop an abdominal brood patch for the purposes of incubation (Pyle 1997). The resulting changes in the skin and vasculature allow for more efficient heat transfer to the clutch. In many cases these are reliable characters. Males in the vast majority of species do not help brood their young and so do not form a brood patch. Alternatively, females have been documented to undergo some cloacal swelling, but it is generally recognized that this only occurs alongside a full brood patch (Pyle 1997). Thus males are sexed by the presence of the cloacal protuberance and females by the presence of a brood patch, while individuals showing no obvious reproductive characters remain unsexed.

Sexing birds by means of reproductive morphology is a common practice among migration monitoring stations of North America and Europe due to its simplicity and lack of expense (Pyle 1997, Svensson 1992). The applicability of using these characters

becomes apparent given the frequency of species that can be sexed by no other means. For example, the sexes are indistinguishable in approximately 43% of the species trapped that breed locally at our study site in southern Ontario. Thus a significant number of birds undergo sexing based on reproductive characters alone. The ability to assign birds to a sex is important to ornithological studies as well as general banding practices. Knowing the sex of migrating passerines allows one to estimate demographic parameters, breeding phenology or characterize migratory patterns, for example.

Although there are alternative methods of sexing passerines, such as laparotomy and genetic sexing, these methods are not applicable on the scale required by migration monitoring stations. By comparison, visual inspection of reproductive characters can be carried out quickly and with little stress to the bird. Given the functional morphology of the male cloacal protuberance and the female brood patch (in species where males do not assist in incubation) classification should be an error free exercise. But how often are mistakes made in the assignment of sex, and how can this be determined in monomorphic species where there are no alternative external features that can be used in sexing? To address this issue, I assessed the accuracy of the technique by comparing morphological sexing by experienced personnel to the results from genetically sexed Song Sparrows (*Melospiza m. melodia*). Song Sparrows are sexually monochromatic, and many females overlap males in size estimates, despite the sexes showing size dimorphism. For these reasons, caution requires that experienced banding personnel should sex this species using reproductive characters. I collected feathers from birds trapped at a migration monitoring station for genetic sexing and to allow us to compare to cautionary sexing of Song Sparrows based on reproductive characters.

Materials and Methods

Song Sparrows (*Melospiza m. melodia*) were trapped at Ruthven Park, Cayuga, Ontario, Canada (N079° 52' 29.4'', W42° 58' 42.7'') in spring 2003 and 2004. Ruthven is part of the Haldimand Bird Observatory and is a migration monitoring station following the guidelines of the Canadian Wildlife Service for migratory passerines (Canadian Wildlife Service 1977). Located in southern Ontario, it is adjacent a large river with a substantial floodplain providing excellent breeding habitat for Song Sparrows. Surrounding farmland and a large intact tract of wet Carolinian forest also provides breeding habitat. Mist nets were placed centrally in these habitats and monitored between mid-March and mid-June by multiple banders, varying in experience from 3 to over 10 years.

Standard 30mm mesh mist-nets were opened prior to sunrise and remained open for approximately 6 hours each day during the banding period when weather was suitable. Birds were retrieved to a banding lab where they were banded, measured and sexed. Unflattened wing chord was taken to the nearest millimeter using a stopped metal ruler ($\pm 0.5\text{mm}$) and mass was measured using an Ohaus Scout SCD-010 balance ($\pm 0.01\text{g}$).

Feather samples were collected for genetic sexing. The outer retrices (left and right r6) were pulled and refrigerated until genetic analysis. We used the P8 and P2 primer pair outlined in Griffiths *et al.* 1998 and shown to be accurate across most Orders of birds including Passeriformes. The Chromo-Helicase-DNA-binding gene (CHD) is highly conserved but the primers amplify across an intron that differs in size between the two sex chromosomes (Z and W). Gel electrophoresis reveals two bands in females

(ZW) and a single band in males (ZZ). When sample quality was in question, results were corroborated using a second primer pair (see Fridolfsson and Ellegren 2000). Individuals that could not be sexed with complete confidence were removed from the sample (9 were eliminated from the total of 72 males and females).

In the hand, Song Sparrows appear monomorphic to the human eye. Nice (1937) sexed Song Sparrows in her study area, in part, by wing chord length supplemented with behavioural observations. This Ohioan population of Song sparrows appears to have a relatively large degree of size dimorphism compared to other populations. Mean male wing chord was 66.3mm (range 62-69mm) compared to 62.1mm in females (range 58-66mm). Despite overlapping through almost 50% of the observed size range, the size distributions are fairly discreet and appear bimodal. At 64mm, 17 birds were sexed as females and 8 as males, with the frequency of overlap dropping significantly at the tails of the overlapping range (where there was only 1 individual of ~40). Similarly, Arcese and Smith (1985) report that Song Sparrows on Mandarte Island could be sexed reliably 90-95% of the time using size. Their method employing discriminant function analysis on mass, flattened wing and keel length however is not appropriate for large scale banding practices. Furthermore the error rate of 5-10% in both cases is potentially misleading since it reflects the error rate across all birds. The actual error rate for the cohort that lies within the range of overlap is much higher which means that a large proportion of birds in this region are inaccurately sexed. This may be biologically significant if it means that the largest females and the smallest males are misidentified or are absent from the sample.

In the population I examined, Song Sparrows appear to show less size dimorphism (see Chapter 2) than the two cases above but are consistent with published accounts. In the subspecies account for *Melospiza m. melodia*, Pyle (1997) reports the wing chord range as 57-71mm with the sexes overlapping between 61-68mm. The size range for all Song Sparrows trapped during 2003-2004 (n=103) was 59-69mm, which is the same for the sub-sample that was genetically sexed (n=63). The results from the genetic sexing revealed that males and females overlap between 62-67mm which is consistent with Pyle (1997). In my population this wing chord range coincides with over 80% of the birds trapped (compare with Nice 1937 and Arcese and Smith 1985 above). Despite showing sexual size dimorphism (males are larger than females; $P=0.025$), the degree of overlap does not facilitate sexing on the basis of size in our population. Similarly, males are heavier than females ($P=0.002$) but the variance displayed by our males was completely overlapped by the range of female weights such that the lightest and heaviest birds trapped were female.

Birds were sexed following the criteria in Pyle (1997). Male passerines can be distinguished by a swelling of the cloaca. Sexually active males begin to develop a cloacal protuberance shortly after they arrive on the breeding grounds in order to facilitate sperm storage and copulation. The swelling enlarges to a maximum coinciding with egg laying in females. The cloacal swelling increases the diameter of the cloaca around the vent as it distends further from the body. In its most mature form, the tip has swollen beyond the base to appear as a bulbous structure. Female breeding morphology usually begins to develop shortly before egg-laying begins. Abdominal feathers are lost prior to the skin engorging with fluid and vascularization of blood vessels in the area of

the abdomen. The fully formed brood patch appears wrinkled compared to adjacent skin and is usually greyish with apparent blood vessels. Males and females were assessed by blowing the abdominal feather tracts aside to inspect the cloacal/abdominal region. Birds were sexed male by the presence of a cloacal protuberance and females by the presence of a brood patch. Birds cannot be sexed by a lack of a reproductive character (i.e. birds were not sexed female by the lack of a protuberance). As mentioned, passerines usually do not possess or arrive on the breeding grounds with reproductive characters therefore the individuals used in this study were re-trapped at various times between April and June of 2003 and 2004.

I assessed whether errors in sexing occurred by comparing the results of the genetic analysis to banding records. A bird was considered improperly sexed when the diagnosis by banding personnel repeatedly (at least twice) and consistently (to eliminate judgment issues) disagreed with the genetic results.

Error rate was assessed using a binomial test and t-tests were used to assess differences between groups as they were normally distributed. All statistical tests were carried out using S-Plus 6.1.

Results

In total, I obtained genetic results from 63 birds from which 28 of those manifested breeding characters that were used for sexing by banding personnel. Banders did not feel confident sexing 35 of the birds based on breeding morphology. Relatively few birds were sexed based on reproductive characters likely because the breeding population tends to be more sedentary when the characters are present; males with

cloacal swellings are typically occupied with territorial defense and females with brood patches are incubating. The latter reason explains why only 8 birds from this sample were females sexed by the presence of a brood patch. The remaining 20 were sexed male by cloacal protuberance.

My sample of genetically sexed Song Sparrows is composed of birds sexed as male, female and those unsexed. Female (based on the presence of a brood patch) wing chords ranged between 61-65mm. Male (based on the presence of a cloacal protuberance) wing chords ranged between 63-67mm while unsexed birds (displaying no evidence of reproductive characters) ranged between 59-69mm.

Eight birds were incorrectly sexed; all were female birds sexed as males based on cloacal morphology. The proportion of these cloacal females (8 mistakes in 28 birds; 29%) significantly differs from the random assignment of birds to a sex (Binomial test, $P=0.035$).

Females with brood patches were observed as early as mid-April, and were trapped uncommonly throughout the breeding season. Similarly cloacal protuberances were present in late March in males, and were continuously caught throughout the spring. Errors in sexing cloacal females occurred beginning in early April until May 18. Thus cloacal females were mistaken as males while other females had brood patches and were incubating, and this occurred relatively late into the breeding season.

Comparing all genetically sexed females ($n=35$) to the group of cloacal females ($n=8$) revealed that the cloacal females were significantly larger (65.5mm; wing chord) than the average female (63.08mm; $P=0.002$). The size range of all females genetically sexed was 59-67mm while the size range of cloacal females was 64-67mm. Using all

genetically sexed females includes birds that did not display any reproductive characters. If these birds breed farther north and are larger than the local birds, it may introduce bias into the results. I compared known local birds (sparrows re-trapped during the breeding season) with those that were not re-trapped during the breeding season (potentially non-local birds). A t-test on wing chord ($P=0.16$) revealed that there were no differences so the two groups of females were combined to increase the sample size.

Discussion

A significant proportion of species trapped at migration monitoring stations is monomorphic to the human eye and therefore must undergo sexing based on the presence of a cloacal protuberance or brood patch. At my site, almost half of the species require these techniques for sexing which may pose a problem if mistakes are common. In comparison to genetic techniques, the results of our study demonstrate that errors can be made in the assignment of sex based on reproductive characters. In all eight cases of misidentification females were called males based on cloacal morphology. Swelling in the area of the cloaca has been documented in female passerines, however this usually occurs alongside a brood patch (Pyle 1997) and so should not result in sexing errors. Given the obviousness of a brood patch it is not surprising that males were not mistakenly called females.

Only a single account could be found detailing the error rate in sexing birds based on cloacal protuberances. Nicholls (2000) reports on the frequency of mistakes in sexing adult Sand Martins using cloacal morphology, verified using genetic techniques. Birds were sexed either male or female based on the measured volume of their cloacal protuberance. In 18 of 51 cases (35%), birds were assigned to the wrong sex even though

males express cloacal swellings averaging twice as large as females. Female Sand Martins appear to have limited variation in the size of the cloacal protuberance ($39.5\text{mm}^3 \pm 9.4$) while male cloacal protuberances vary greatly ($79.4\text{mm}^3 \pm 31.2$) due to seasonal effects. Although no details were available, it would appear likely that, due to the degree of variation, a large proportion of the mistakes were males (having small cloacal protuberances) being classified as females. The author suggests using a combination of cloacal protuberance and brood patch to improve reliability, however in our case the majority of the population showed neither character. In a scenario such as this, most birds are categorized on the basis of the degree of cloacal swelling; birds that appear to have large cloacal swellings are male, while females and males with no apparent swelling remained unsexed (the majority of the sample).

Despite using brood patches and cloacal protuberances, errors occurred in 29% of the cases where sparrows were sexed based on reproductive characters, which is consistent with Nicholls (2000). However this does not mean that cloacal protuberances are misinterpreted 29% of the time; in reality the ability to properly examine a bird and assess whether it has a cloacal protuberance is better than this. The majority of females remained unsexed because so few brood patches were present, but this means that they were properly assessed as having no cloacal protuberance. Including all females, mistakes were made in only 15% of cases where cloaca were examined.

I found that larger females were more likely to be mistaken for males based on cloacal morphology. One argument explaining this result is that wing chord was used to aid in identification. Given the published overlap in size of the sexes (61-68mm; Pyle 1997) as well as the overlap determined from our genetic results (62-67mm; 81% of the

population) sexing birds based on wing chord is not accurate and avoided. The size range of cloacal females was 64-67mm which coincides with this overlap region. Furthermore, there were numerous large birds having wing chords of 67, 68 and 69mm that were likely males but remained unsexed by banding personnel. Likewise, small birds (59-60mm wing chord), presumably females, also remained unsexed when there were no signs of reproductive characters. This implies that cloacal females were indeed larger and that this relationship was not confounded by the use of wing chord in identification of sex.

There may be several reasons explaining why these females were mistaken for males. Cloaca of larger females may have a greater likelihood of resembling a male's cloaca. In large females, the size of the cloaca may appear larger when size is gauged relatively. In my case, cloacal protuberances were assessed on the basis of size and shape rather than empirically measured (however errors can still be made in the latter, see Nicholls (2000)). Assessing birds on an individual basis using relative size of the cloaca could be the source of the mistakes.

Interestingly, there are anecdotes of female Song Sparrows possessing other characteristically male traits. Nice's (1937) observations of Song Sparrows in Ohio revealed that a small proportion of the females in her population regularly called. These females sang similar territorial songs from an exposed perch as did males, less musically, but at a rate typical of a motivated male. Two of these birds proved quite antagonistic, initiating aggression and chasing foreign males out of the territory (usually reserved as a male duty). In a single case, one female partook in an elaborate postural display only ever witnessed in males defending their territories. Calling behaviour in females has been witnessed in other accounts as well (Arcese *et al.* 1988, Saunders 1929, Wetherbee

1935). Wetherbee (1935) reported on a female that called repeatedly in April through to June 17. This coincides with the period that females were mistakenly sexed as males.

In some instances of sexual transference in other species, even more obvious traits may be displayed by females, such as male plumage (Bleiweiss 2001, Tella *et al.* 1997) or in extreme cases, full cloacal protuberances (Nakamura 1990). In this latter case, the observation of cloacal swellings in a related species evidence that females are capable of producing cloacal protuberances morphologically similar to those of males but via a different physiological mechanism.

A potential mechanism explaining elevated aggression, singing and cloacal swellings in large females could operate via testosterone. Testosterone has been offered to explain female calling in Song Sparrows (Arcese *et al.* 1988) and females administered testosterone sing more often (Wingfield 1994). In other species, females with higher testosterone titres are observed to be more aggressive (Langmore *et al.* 2002). In our sample of Song Sparrows, we have no observational data to support this.

My results show that potentially unavoidable errors in sexing can be made by experienced banders. I am uncertain of the breadth of this phenomenon as a generalization across species, however Nicholls (2000) supports that this could be a regular phenomenon. From a migration monitoring perspective, this may imply small overestimates in the occurrence of males. From the point of view of future research, the biological implications may prove interesting. My results may support the idea that in a portion of the population, the appearance of female cloacal morphology can resemble that of males. In my example this equates to 19% of females (8 females sexed incorrectly as male based on cloacal protuberance out of 43 genetically sexed females) that may

manifest confusing characteristics. That the variation in female cloaca may overlap that of males is substantiated by Nicholls (2000). Despite being considered distinct (Pyle 1997), male and female cloacal swellings may overlap in a potentially large number of species confounding attempts to accurately sex birds based on reproductive characters.