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Population Demographics and Genetics of Spix's Disk-Winged Bat: Insights Regarding Survival, Mate Choice, Gene Flow and Effective Population Size

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POPLATION DEMOGRAPHICS AND GENETICS OF SPIX’S DISK-WINGED BAT: INSIGHTS REGARDING SURVIVAL, MATE CHOICE, GENE FLOW AND EFFECTIVE POPULATION SIZE

by

Michael R. Buchalski

A dissertation submitted to the Graduate College in partial fulfillment of the requirements for the degree of Doctor of Philosophy Biological Sciences Western Michigan University August 2013

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Simultaneous study of the demographics and genetics of populations are relatively rare within the literature, despite insights their combined use offers regarding the life history, ecology, and evolution of species. Here I take a comprehensive approach, using capture-recapture data, polymorphic microsatellite markers, and various modeling techniques to examine the demographics and genetics of multiple local populations of Spix’s disk-winged bat (*Thyroptera tricolor*) in southwestern Costa Rica. *T. tricolor* is a highly gregarious, neotropical bat species known to form kin-based social groups with high retention of offspring of both sexes. The implications of this highly unusual social structure for survival, inbreeding, and population genetic structure were previously uninvestigated. Capture-recapture modeling based on 5 years of data at three sites indicates survival probability in *T. tricolor* is strongly age-structured, with three stages: juveniles (0.55 [95% CI = 0.41–0.68]), prime-age adults (0.77 [0.62–0.87]), and senescent adults (0.45 [0.38–0.52]). Survival probabilities vary significantly among populations as well, suggesting strong effects of site-specific environmental factors. Using genetic pedigree reconstruction and roosting home range mapping, I examine the effects of mate choice on inbreeding avoidance and gene flow. Analyses indicate that despite sharing small home ranges (~ 0.2 ha) with close relatives, *T. tricolor* mate outside
of their kin-based social groups with individuals separated by large distances (~ 500 m). Such a pattern of mate choice has the effect of dispersing alleles, despite low juvenile dispersal from natal patches. I further evaluate the potential for a polygynous mating system in this species by comparing genetic and demographic estimates of effective population size $N_e$. Genetic estimates indicate $N_e$ is significantly lower than adult population size $N$ with $N_e/N$ ratios of 0.28–0.42. Demographic modeling based on capture-recapture data suggests low $N_e$ values are the result of few males (< 10%) achieving reproductive success, consistent with polygyny. Utilizing a comprehensive approach to study the demographics and population genetics of this species provides insights into life history characteristics that would not otherwise be possible, including strong age-structured survival, effects of mate choice on inbreeding avoidance, and the influence of polygyny to reduce genetic diversity within populations.
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CHAPTER I

CAPTURE-RECAPTURE EVIDENCE FOR SPATIAL AND AGE-STRUCTURED VARIANCE IN SURVIVAL IN SPIX’S DISK-WINGED BAT
(*THYROPTERA TRICOLOR*)

Introduction

The study of life histories encompasses all significant features of the life cycle of an organism, particularly as they relate to survival and reproduction (Stearns 1992). Much of the diversity of life histories of mammals can be explained by the fast-slow continuum (Bielby et al. 2007; Gaillard et al. 1989; Promislow and Harvey 1990), with long-lived species having late ages of first reproduction, high survival, and low reproductive output (i.e. slow species), and fast species showing the converse. Adult survival is the major determinant of population growth among slow species (Gaillard and Yoccoz 2003), making estimates of survival rate and identification of the factors affecting its variation vital to understanding the population dynamics of such species.

Bats (Chiroptera) have the typical features of slow species within the fast-slow continuum of life histories. Unlike many small-bodied mammals, bats have high survival rates (e.g. O'Shea et al. 2004; Papadatou et al. 2009; Schorcht et al. 2009), long lifespans (Brunet-Rossinni and Austad 2004; Jones and MacLarnon 2001), and low reproductive outputs (Barclay and Harder 2003). Bats constitute a quarter of all mammal species (over 1,200 world-wide), with the majority occurring in tropical regions (Findley 1993). Bats are declining globally (Mickleburgh et al. 2002) and ~ 50% of microchiropteran species are considered imperiled or data deficient (Hutson et al. 2001). Adult bat survival is
known to be of major importance for population dynamics (O'Shea et al. 2011; Pryde et al. 2005), yet little is known about typical survival probabilities. Basic demographic data obtained by reliable methods are available for relatively few temperate species (O'Shea et al. 2004), while data for neotropical bats are lacking. Identifying factors affecting survival would provide valuable information about how tropical bat populations respond to anthropogenic changes to landscapes and habitat, and inform conservation and management strategies (Pryde et al. 2005).

Variance in vital rates is often linked to spatiotemporal variability in habitat availability or quality, and existing research suggests such factors play an important role in the survival of bats. Seasonal variation in conditions have been shown to affect reproduction and survival of temperate bat species (Frick et al. 2007; Frick et al. 2010; O'Shea et al. 2011), presumably through changes in insect abundance (Frampton et al. 2000). Other studies have demonstrated bat survival can vary with winter temperatures for hibernating species (Gerell and Lundberg 1990; Hoyle et al. 2001; Pryde et al. 2005). Spatial differences in habitat quality (e.g., predation, distances to foraging areas, roost availability, etc.) also have implications for bat survival, and reliable estimates of vital rates likely requires sampling multiple populations (Frederiksen et al. 2005). Yet comparative studies of variance in survival probabilities among bat populations are uncommon (Papadatou et al. 2011), with most sampling only 1 or 2 roosts (O'Shea et al. 2004).

Measuring age-specific survival rates can help identify different age-related mortality risks, demonstrating the relative importance of certain life stages to overall population dynamics (Morris and Doak 2002). Knowledge about age-structured survival
in bats is limited, with most accounts indicating first-year survival is lower than subsequent years (i.e. juvenile and adult; Frick et al. 2007; Frick et al. 2010; Hoyle et al. 2001; O'Shea et al. 2010; Pryde et al. 2005; Schaub et al. 2007). This pattern of low first-year survival is often attributed to greater juvenile vulnerability to predators, or a failure to gain sufficient energy stores for growth, development, and in the case of temperate bats insufficient fat stores for first hibernation. Complicated age-structure typified by three age classes is typical of mammals, including juvenile, adult, and senescent stages (Caughley 1966). Age-structure with more than two age classes has not been supported in bats, but was examined in only two studies (Schortch et al. 2009; Sendor and Simon 2003).

Sex differences in survival rate can have direct effects on population size (Garrott 1991; Larsen et al. 1989) and the evolution of mammalian reproductive strategies (Partridge and Harvey 1988; Stearns 1992). In most mammalian species, female lifespan exceeds males (e.g. Clutton-Brock and Isvaran 2007; Owens 2002), with this pattern considered indicative of the cost of sexual selection paid by males for traits that enhance reproductive success (Moore and Wilson 2002). Within bats, there is evidence of higher survival among females (Boyd and Stebbings 1989; Gerell and Lundberg 1990; Hoyle et al. 2001; Pryde et al. 2005), while others found no difference (Papadatou et al. 2009; Schaub et al. 2007; Sendor and Simon 2003). Identifying sex-based differences could be particularly significant, as adult female survival likely represents an important determinant of bat population dynamics.

Capture-recapture (CMR) methods have been among the most important for the study of life history, particularly of cryptic animals (Lebreton et al. 1992; Pollock et al.
Capture-recapture has been applied in bats to track movements between sites (Fleming and Eby 2003), to study social systems and population structure (e.g., Chaverri 2010; Entwistle et al. 2000; Vonhof et al. 2004) and to estimate population size (Rivers et al. 2006; Vonhof and Fenton 2004). The development of sophisticated models and flexible software (White and Burnham 1999) has made it possible to estimate survival and recapture rates for predefined groups or age-classes, providing researchers with a powerful tool for understanding factors associated with population dynamics, ecology, and behavior of animal populations (Lettink and Armstrong 2003). An increasing number of modern ecological studies of bats have used these modeling techniques to systematically test hypotheses about sources of variation in vital rates, yet such techniques have yet to be applied to a neotropical species.

Spix’s disk-winged bat (*Thyroptera tricolor*) is a neotropical species that roosts within the highly ephemeral, developing leaves of *Heliconia* and *Calathea* plants (Wilson and Findley 1977). This bat displays all-offspring philopatry, forming social groups consisting of one or more reproductive females and offspring of both sexes from multiple years (Chaverri and Kunz 2011). *T. tricolor* show a pattern of reproductive seasonality common to many neotropical insectivorous bat species. However, *T. tricolor* has an unusually slow life history compared to other tropical bats, which may be attributable to its unique roosting ecology and social behavior. Females have long gestation and lactation periods lasting approximately half the year in total (Chaverri and Vonhof 2011). Females produce a single pup per year, pups are born small, and juvenile mortality is high, with ~30% of young dying before age six months. Postnatal growth and development are slow, and time to sexual maturity in females is longer than most tropical
bat species, resulting in distinct juvenile, subadult, and adult age classes. *T. tricolor* therefore belong to the slower lane of the slow-fast continuum of life history variation in bats (Barclay and Harder 2003), and estimates of vital rates for this bat are currently unavailable.

We were interested in how the unique ecological and behavioral traits of *T. tricolor* affect variation in survival throughout the annual seasonal and reproductive cycle, and as bats develop and age. The aforementioned life history traits suggest the potential for age- and sex-based variation in survival rates. Using a Cormack–Jolly–Seber model, we analyzed capture-recapture data spanning 5 years on 245 individual *T. tricolor* from three banded populations in southwestern Costa Rica to study variation in monthly survival probabilities. Our data set is unique in that it makes comparison of three natural populations of tropical bats, while examining variation in monthly survival within the annual cycle. We aimed to identify ecological and life history factors affecting survival by assessing differences between: (1) populations, (2) wet vs. dry seasons, (3) male and female reproductive periods, and (4) age-classes.

In addition, previous studies of bat longevity indicate non-hibernating tropical species have shorter lives than hibernating, temperate bats (see Barclay and Harder 2003; Wilkinson and South 2002). However, the use of maximum longevities for demographic inference has been criticized, with the application of survival rate estimates viewed as a much more appropriate comparative life-history parameter (Krementz et al. 1989). We calculated mean lifespan from survival rates for *T. tricolor*, generating a more demographically representative measure of longevity for this tropical bat and allowing for comparisons to previously published values for temperate species.
Methods

Study species

*T. tricolor* is a small (3–4 g) insectivorous bat found in lowland neotropical forests from southern Mexico to southeastern Brazil (Wilson and Findley 1977). This species is morphologically specialized for roosting in furled, developing leaves of members of the order Zingiberales (primarily in the genera *Heliconia* and *Calathea*), which are suitable as roosts for ~1 day (Findley and Wilson 1974; Vonhof and Fenton 2004). The reproductive period of both sexes is unusually long for tropical bats (Chaverri and Vonhof 2011). Males with enlarged testes are commonly observed during the 5 months between August and December. The gestation period for females is ~3.5 months, with pregnant individuals easily recognizable in hand by December. Parturition of a single pup occurs between February and March and lactating typically begins in February, lasting ~4 months. Parturition coincides with the beginning of the wet season, during increases in insect abundance (Janzen 1973; Richards and Windsor 2007). Offspring mortality is high, and ~30% of young die before age 6 months, the majority of those occurring within the first 3 months. Young are capable of sustained flight at 2 months of age, and attain adult body size by 6 months of age. Before juveniles achieve flight, females must transport their young between roosts on a daily basis, which may be particularly costly for mothers and dangerous for young. Following the onset of flight, young *T. tricolor* may still be prone to mortality due to starvation resulting from inefficient foraging, and predation or exposure resulting from inexperience locating roosts on a daily basis. Conversely, once offspring can fly survival may be enhanced by cooperation with other group members, since social groups in *T. tricolor* maintain contact
while locating roosts (Chaverri et al. 2010). Males attained sexual maturity earlier than females, at one year of age, while females become reproductive after their first year.

**Study sites and data collection**

This study was conducted at three sites located in southwestern Costa Rica, from October 2006 through April 2010 (Fig. 1). The three sites, Finca (8°38’ N, 83°05’ W), Esquinas (8°42’ N, 83°12’ W), and Ureña (8°40’ N, 83°12’ W) consist of primary and late secondary forest, with *Heliconia imbricata* and *Calathea lutea* plants dominating the understory. These three sites were selected based on the density of furled leaves and recapture rates high enough to facilitate capture-recapture modeling (Table 1). Mean distance between sites was 11.1 km, with a range of 4.3–15.8 km (Fig. 1). Weather patterns in the region are distinctly seasonal, including wet (May-October) and dry (November-April) seasons (Coen 1983).

![Figure 1. Location of three Spix’s disk-winged bat (*Thyroptera tricolor*) local populations in southwestern Costa Rica where capture-recapture data were collected from 2006 to 2010.](image-url)
Table 1. Number of *T. tricolor* banded, number (and percentage) of bats recaptured, total number of recaptures (including individuals repeatedly recaptured) at three sites in southwestern Costa Rica, from 2006 to 2010

<table>
<thead>
<tr>
<th>Site</th>
<th>Banded (n)</th>
<th>Recaptured</th>
<th>Total recaptures</th>
<th>Avg. recaptures per bat (min, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finca</td>
<td>66</td>
<td>49 (74%)</td>
<td>474</td>
<td>7 (0, 23)</td>
</tr>
<tr>
<td>Ureña</td>
<td>65</td>
<td>46 (71%)</td>
<td>253</td>
<td>4 (0, 21)</td>
</tr>
<tr>
<td>Esquinas</td>
<td>114</td>
<td>70 (61%)</td>
<td>282</td>
<td>2 (0, 13)</td>
</tr>
</tbody>
</table>

Capture-recapture surveys were conducted August 2006 through April 2010 within a pre-established area (Finca = 0.78 ha, Ureña = 0.81 ha, Esquinas = 3.30 ha) at each site. Surveys occurred every 2 months on average, during both wet and dry seasons, as well as reproductive vs. non-reproductive periods for both sexes. Surveys increased in frequency to once every 2 weeks during the parturition period to facilitate marking individuals of known age. Bats were captured at roosts by pinching the top of the leaf and directing them into a cloth holding bag. Bats were fitted with individually numbered metal wing bands (Porzana, Inc.), sexed, aged, and their reproductive condition assessed (Anthony 1988; Racey 1988). All bats were released shortly after handling. Each bat was assigned to one of three age-classes: juveniles (i.e. young-of-the-year), subadults, and adults. Bats were classified as juveniles if their dorsal pelage was gray, if we observed the presence of cartilaginous epiphyseal plates in metacarpals and phalanges, or if the individual was suckling. The subadult age category was limited to second-year females, as males reach reproductive maturity within one year. Subadult females are easily distinguished as they show no signs of reproductive activity, such as a bare patch surrounding enlarged, keratinized nipples. Bats were classified as adults if there was
evidence of current (i.e., enlarged testes, pregnancy, or lactation) or previous (i.e., testicular descent, keratinized nipples) reproductive activity, but exact age was unknown.

Recaptures indicated no movement between sites, so we considered each site to represent a separate population and made no attempt to incorporate movement into our analysis (i.e. multistate models). Individual encounter histories were created coding each bat as either 1 (encountered) or 0 (not encountered) during each of 28 survey occasions. Data were divided into 15 groups based on age, sex, and site. We created encounter history files for both monthly and annual time intervals.

Model parameterization and goodness of fit testing

We used mark (White and Burnham 1999) to fit competing models of monthly apparent survival rate $\phi_{mo}$ and recapture rate $p_{mo}$ based on extensions of the Cormack–Jolly–Seber (CJS) open-population capture-recapture model (Lebreton et al. 1992). We defined monthly apparent survival $\phi_{mo}$ as the probability an animal is alive and remains in the study area available for recapture during a monthly (30-day) time period. Monthly recapture probability $p_{mo}$ was defined as the probability a marked individual that is alive and in the population during a monthly time period is captured during that period.

The CJS model has several assumptions that can be violated by transience, trap dependence, or mark loss (Pollock et al. 1990). The CJS model cannot distinguish mortality from permanent emigration, so if significant movement from the study area occurs, estimates of survival rates will be biased low. We assume that permanent emigration was minimal in our study system, as previous studies have demonstrated high natal philopatry and fidelity to small roosting home ranges in this species (Chaverri and Kunz 2011; Vonhof et al. 2004). We also assume that unknown mark loss was negligible,
as forearm bands are more permanent than most other marking systems applied to vertebrates (Keen 1988). To test whether our data met the assumptions of the CJS model, we conducted goodness-of-fit (GOF) testing on the fully time-dependent (i.e. global) model. We used Tests 2 and 3 in RELEASE (Burnham et al. 1987) for heterogeneity in survival (transience effect; Pradel et al. 1997) and recapture probabilities (trap dependence effect; Pradel 1993). The sum of the $\chi^2$ components of both tests provides a general test of the adequacy of the global model. To determine if our data were overdispersed, we calculated the variance inflation factor, $\hat{c}$ as the ratio between the $\chi^2$ value from the GOF test and its degrees of freedom (Anderson and Burnham 1999) and scaled model deviances appropriately.

Following GOF testing, a set of alternative models containing fewer parameters was developed. Each model represented a specific hypothesis regarding the effect of site, time, sex, reproductive condition, and age on bat survival and recapture. To simplify the model selection process, we first constructed models which held survival rate constant and allowed monthly recapture $p_{mo}$ to vary according to different factors. A priori models on $p_{mo}$ included constant recapture ($.$), full time-dependence ($t$), time-dependence constrained by season (wet vs. dry), reproductive period, and age, and group effects of site and sex (Table 2). Models on $p_{mo}$ included main effects, as well as additive and interactive effects when their inclusion represented biologically plausible hypotheses. Model notation follows the approach recommended by Lebreton et al. (1992), including the parameter modeled ($\phi_{mo}$ or $p_{mo}$) followed by a code indicating the effects tested. Model selection was performed by minimizing Akaike’s Information Criterion corrected for small sample size ($AIC_c$; Burnham and Anderson 2002). For model ranking we
reported AIC$_c$ differences ($\Delta_i$; difference in AIC$_c$ score between ith and top-ranked model) and Akaike weights ($w_i$; probability that the ith model is the best approximating model among candidate models). Models with $\Delta$AIC$_c$ $<$ 2 were considered to have strong support from the data, and models with $\Delta$AIC$_c$ $>$ 10 were considered to have no support (Burnham and Anderson 2002). The best-fit model parameterization of $p_{mo}$ was then used in all subsequent models of apparent monthly survival $\phi_{mo}$ (see below).

Table 2. Summary of model selection results for monthly recapture rate $p_{mo}$ of *T. tricolor* in southwestern Costa Rica, 2006-2010. Models ranged by ascending $\Delta$AIC$_c$, those with a $\Delta$AIC$_c$ $<$ 10 are shown in bold.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC$_c$</th>
<th>$\Delta$AIC$_c$</th>
<th>$w_i$</th>
<th>Model likelihood</th>
<th>$np$</th>
<th>Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 $p_{mo}$(site $\cdot$ $t$)</td>
<td>2658.2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>82</td>
<td>2481.9</td>
</tr>
<tr>
<td>2 $p_{mo}$(site + $t$)</td>
<td>2796.3</td>
<td>138.1</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>2734.7</td>
</tr>
<tr>
<td>3 $p_{mo}$(t)</td>
<td>2873.7</td>
<td>215.5</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>2816.3</td>
</tr>
<tr>
<td>4 $p_{mo}$(rep period $\cdot$ site)</td>
<td>2942.8</td>
<td>284.7</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>2916.5</td>
</tr>
<tr>
<td>5 $p_{mo}$(season $\cdot$ site)</td>
<td>2950.7</td>
<td>292.6</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>2936.6</td>
</tr>
<tr>
<td>6 $p_{mo}$(sex $\cdot$ site)</td>
<td>2955.8</td>
<td>297.7</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>2941.7</td>
</tr>
<tr>
<td>7 $p_{mo}$(site)</td>
<td>2956.8</td>
<td>298.7</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>2948.8</td>
</tr>
<tr>
<td>8 $p_{mo}$(age $\cdot$ site)</td>
<td>2957.9</td>
<td>299.7</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>2937.7</td>
</tr>
<tr>
<td>9 $p_{mo}$(season)</td>
<td>3017.2</td>
<td>359.1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3011.2</td>
</tr>
<tr>
<td>10 $p_{mo}$(age)</td>
<td>3023.5</td>
<td>365.3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>3015.5</td>
</tr>
<tr>
<td>11 $p_{mo}(.)$</td>
<td>3024.1</td>
<td>366.0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3020.1</td>
</tr>
<tr>
<td>12 $p_{mo}$(sex)</td>
<td>3025.2</td>
<td>367.1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3019.2</td>
</tr>
<tr>
<td>13 Global model</td>
<td>6991.1</td>
<td>4332.9</td>
<td>0</td>
<td>0</td>
<td>810</td>
<td>1940.7</td>
</tr>
</tbody>
</table>

AIC$_c$ = Akaike’s information criterion, $\Delta$AIC$_c$ = difference in AIC$_c$ between each model and the best approximating model, $w_i$ = Akaike’s weight, $np$ = number of estimable parameters, Dev = deviance. Model notation: $.$ = constant recapture, $t$ = full time-dependence (different values for each survey occasion), rep period = different recapture during reproductive vs. non-reproductive periods. $\cdot$ indicates interaction effects (independent values for each level of each parameter) and $+$ indicates additive models (values for each level of each parameter vary in parallel). Global model was used for the goodness-of-fit test.

A priori models on apparent monthly survival $\phi_{mo}$ fell into three categories, testing the effects of (1) site and time, (2) sex and reproductive condition, and (3) age on survival (Table 3). Models of site and time included constant survival ($.$), full time-dependence ($t$), time-dependence constrained by season, the main effect of site, and additive and interaction effects of site and time (models 16 to 23, Table 3). For age
structured models, we focused on key developmental stages which might experience
different survival rates than adults, and introduced an age effect which was constrained to
end after the appropriate amount of time following birth. We modeled pup survival for
both the first 3 and 6 months of life (models 26 and 28). The majority of pup mortality
occurs within the first 3 months, and by 6 months pups are weaned, largely independent,
and have reached 100% of adult size. The model including a 6 month pup age-class
showed less support from the data, so all subsequent models of age structure included the
3 month pup age-class. We sequentially parameterized increasingly complex age
structure into this model, including a 9 month juvenile stage for the remainder of the first
year (model 27), a second subadult year (model 25), and a model of full age structure
(model 24), which included pup and juvenile stages followed by separate survival
estimates for each subsequent year of life. Because the exact age of bats captured as
adults was unknown, their survival probability was estimated separately. Models of sex
and reproductive condition tested whether variation in $\phi_{mo}$ existed between sexes (model
34) or during reproductive vs. non-reproductive periods. We modeled females separately
during both the 3.5 month pregnancy period (model 30) and extended this to include the 4
month lactation period following parturition (model 29). We modeled males separately
during the 5 months they are typically observed scrotal (model 32). We also created
models which included the effects of reproductive condition for both sexes
simultaneously (models 31 and 33).

Models from all three categories were compared using $AIC_c$ (Table 3) and those
with $\Delta AIC_c < 10$ were considered to have reasonable support from the data. These models
were considered further by examining whether combined parameter structures from
different effect categories improved overall model fit. The outcome of combined models is discussed in the Results section.

Table 3. Summary of model selection results for apparent monthly survival rate $\phi_{mo}$ by category, including site and time, age structure, sex and reproductive condition, and combined parameterizations. Models with a $\Delta AIC_c < 10$ are shown in bold.

<table>
<thead>
<tr>
<th>Model</th>
<th>$AIC_c$</th>
<th>$\Delta AIC_c$</th>
<th>$w_i$</th>
<th>Model likelihood</th>
<th>$np$</th>
<th>Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined parameterizations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>$\phi_{mo}(\text{Full age} + \text{site})$</td>
<td>2640.94</td>
<td>0</td>
<td>0.725</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td>15</td>
<td>$\phi_{mo}(\text{Full age} \cdot \text{site})$</td>
<td>2649.72</td>
<td>8.78</td>
<td>0.009</td>
<td>0.012</td>
<td>99</td>
</tr>
<tr>
<td>Site and time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>$\phi_{mo}(\text{site} + \text{season})^\dagger$</td>
<td>2644.64</td>
<td>3.69</td>
<td>0.114</td>
<td>0.158</td>
<td>85</td>
</tr>
<tr>
<td>17</td>
<td>$\phi_{mo}(\text{site})$</td>
<td>2644.95</td>
<td>4.01</td>
<td>0.098</td>
<td>0.135</td>
<td>84</td>
</tr>
<tr>
<td>18</td>
<td>$\phi_{mo}(\text{site} \cdot \text{season})^\dagger$</td>
<td>2646.46</td>
<td>5.52</td>
<td>0.046</td>
<td>0.063</td>
<td>87</td>
</tr>
<tr>
<td>19</td>
<td>$\phi_{mo}(\cdot)$</td>
<td>2658.16</td>
<td>17.22</td>
<td>0</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>20</td>
<td>$\phi_{mo}(\text{season})$</td>
<td>2658.28</td>
<td>17.34</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>21</td>
<td>$\phi_{mo}(t)$</td>
<td>2679.93</td>
<td>38.99</td>
<td>0</td>
<td>0</td>
<td>108</td>
</tr>
<tr>
<td>22</td>
<td>$\phi_{mo}(\text{site} + t)$</td>
<td>2731.97</td>
<td>91.02</td>
<td>0</td>
<td>0</td>
<td>136</td>
</tr>
<tr>
<td>23</td>
<td>$\phi_{mo}(\text{site} \cdot t)$</td>
<td>2733.80</td>
<td>92.86</td>
<td>0</td>
<td>0</td>
<td>162</td>
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<tr>
<td>Age structure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$\phi_{mo}(\text{Full age})$</td>
<td>2650.14</td>
<td>9.20</td>
<td>0.007</td>
<td>0.010</td>
<td>87</td>
</tr>
<tr>
<td>25</td>
<td>$\phi_{mo}(\text{Pup 3mo, Juv, Sub})$</td>
<td>2655.97</td>
<td>15.03</td>
<td>0</td>
<td>0.001</td>
<td>84</td>
</tr>
<tr>
<td>26</td>
<td>$\phi_{mo}(\text{Pup 3mo})$</td>
<td>2659.05</td>
<td>18.10</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>27</td>
<td>$\phi_{mo}(\text{Pup 3mo, Juv})$</td>
<td>2659.44</td>
<td>18.50</td>
<td>0</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>28</td>
<td>$\phi_{mo}(\text{Pup 6mo})$</td>
<td>2660.44</td>
<td>19.50</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>Sex and reproductive period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>$\phi_{mo}(\text{Lac})$</td>
<td>2658.02</td>
<td>17.08</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>30</td>
<td>$\phi_{mo}(\text{Preg})$</td>
<td>2658.93</td>
<td>17.98</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>31</td>
<td>$\phi_{mo}(\text{Scrot, Lac})$</td>
<td>2659.48</td>
<td>18.53</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>32</td>
<td>$\phi_{mo}(\text{Scrot})$</td>
<td>2659.74</td>
<td>18.79</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>33</td>
<td>$\phi_{mo}(\text{Scrot, Preg})$</td>
<td>2660.21</td>
<td>19.27</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>34</td>
<td>$\phi_{mo}(\text{Sex})$</td>
<td>2660.30</td>
<td>19.35</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>13</td>
<td>Global model</td>
<td>6991.08</td>
<td>4350.13</td>
<td>0</td>
<td>0</td>
<td>810</td>
</tr>
</tbody>
</table>

$AIC_c = \text{Akaike's information criterion, } \Delta AIC_c = \text{difference in } AIC_c \text{ between each model and the best approximating model, } w_i = \text{Akaike's weight, } np = \text{number of estimable parameters, Dev = deviance. Model notation: . = constant survival; } t = \text{full time-dependence; Pup, Juv, and Sub indicate age classes for which survival is estimated separately from adults; 3mo and 6mo indicate length of pup age class in months; Lac, Preg, and Scrot indicate survival is estimated separately during the reproductive period for that sex. } \cdot \text{indicates interaction effects and } \dagger \text{indicates additive models. Global model was used for the goodness-of-fit test.}$

$^\dagger \text{Likelihood ratio test indicated the effect of season did not provide significantly better fit than the site only model.}$
Estimating mean life span

We calculated mean lifespan (MLS) of *T. tricolor* using age-specific estimates of survival and the method of Brownie et al. (1985) as,

\[
\text{MLS} = \left\{ \frac{1}{-\ln(\phi_i)} + \frac{\phi_i}{-\ln(\phi_A)} + \frac{\phi_i}{\ln(\phi_i)} \right\}
\]

where \( \phi_j \) is juvenile survival and \( \phi_A \) is adult survival. We used estimates of \( \phi_j \) and \( \phi_A \) from a simplified age-structure model on an annual time-scale (see results). The variance was calculated using the delta method (Seber 1973), details provided in Dinsmore et al. (2003).

Results

Capture-recapture results

We banded a total of 245 bats across three sites over 28 capture occasions from 2006 to 2010. Of those, 165 were captured more than once (i.e. recaptures). We banded 66 bats at Finca, 65 at Ureña, and 114 at Esquinas (Table 1). These bats included 139 adults and 106 individuals of known age (i.e. juveniles and subadults). The percentage of bats recaptured were approximately equal at Finca and Ureña (74 vs. 71%, respectively), and was lowest at Esquinas (61%). Mean, minimum, and maximum number of recaptures per bat per site are given in Table 1.
Goodness-of-fit

Both GOF tests on the global model indicated the data met the assumptions of the CJS model, with no heterogeneity in recapture probabilities due to trap dependence, or in survival probabilities due to transience detected (Test 2 $\chi^2 = 103.5$, $df = 139$, $P = 0.99$; Test 3 $\chi^2 = 38.9$, $df = 79$, $P = 1.00$). This indicates that the models tested provided acceptable fits to the data. The variance inflation factor, estimated as the ratio between the $\chi^2$ and its degrees of freedom ($\hat{c} = 142.32/218 = 0.65$), suggested no overdispersion of the data ($\hat{c} = 1$ if the model fits perfectly). There is lack of consensus on how to address $\hat{c} < 1$, so we followed the advice of Cooch and White (2012) and assumed $\hat{c} = 1$, making no adjustment to model deviances.

Model selection results

A single parameterization (model 1) in which monthly recapture rate $p_{mo}$ varied by a site · time interaction showed 99% support from the data according to $AIC_c$ weights (Table 2). The second best model which considered only the additive effects of site and time had essentially no support from the data. The best-fit model suggests considerable fluctuation in recapture probability between capture occasions and sites, with no effect of age, sex, or reproductive condition on recapture probability. Estimates of recapture probabilities generally ranged from 0.07–0.96, depending on site and capture occasion. All subsequent models of monthly survival $\phi_{mo}$ were constructed with this parameterization for recapture (described below).

A total of nineteen models of apparent monthly survival rate $\phi_{mo}$ were constructed among the three categories of effects, including site and time, sex and reproductive condition, and age. The global model had no support ($\Delta AIC_c >> 10$; $w_i = 0$; Table 3).
Models with site and site by time variation in survival showed good fit when time was constrained as a seasonal effect (models 16–18), and had approximately equal support from the data (i.e. $\Delta AIC_c \approx 2$). Inspection of parameter estimates indicated the effect of time was predominantly the result of a single season within a single year at one site where survival was particularly low. Though an important characteristic of the data, this suggests time-structured models are not particularly informative of the life history of this species, as time-specific trends in monthly survival appear absent. The effect of site was consistent across capture occasions on the other hand. We applied likelihood ratio tests to determine the significance of season as a factor in the model. Models with site and season effects (site + season and site $\cdot$ season) did not provide better fit than the model with the effect of site only ($\chi^2 = 2.63$, $df = 1$, $P = 0.105$ and $\chi^2 = 5.46$, $df = 3$, $P = 0.141$, respectively). Based on these results, and the potential for incorporating site, season, and age structure (see below) into a model resulting in over-parameterization, we chose to retain the effect of site and disregard seasonal effects for final model construction.

The model of full age-structure (model 24) showed support from the data ($\Delta AIC_c < 10$; Table 3), indicating that survival varied significantly with age (i.e. pup, juvenile, each subsequent year after birth, and adults). An unexpected result was that models including the effects of sex and reproductive condition showed no support from the data ($\Delta AIC_c >> 10$; $w_i = 0$), suggesting any such variation was not strong. Therefore, the effects of these factors were not considered further.

We attempted to improve model fit by combining parameterizations of site- and age-structured apparent monthly survival. The best-fit model from the final comparison included the additive effects of age and site (Model 14; Table 3). This model had an
evidence ratio \( w_1/w_2 \) (Burnham and Anderson 2002) of 6.4:1 over the second highest ranking model (model 16), one which did not include age structure. The additive model suggests age-structured variation in survival is consistent among populations (i.e. varies linearly on a logit scale). The best-fit model had a weight of 0.73 and was 3.69 \( \Delta AIC_c \) units above the next highest ranking model. A ranking of relative variable importance (Burnham and Anderson 2002) among models which showed reasonable support from the data (i.e. \( \Delta AIC_c < 10 \)) indicated that site (0.99) is the most important variable, age (0.73) is second in importance, and season (0.16) is third.

**Survival estimates**

Estimates of apparent monthly survival rates \( \phi_{mo} \) from the best-fit model illustrate the magnitude of age- and site-specific differences (Fig. 2; Table 4). Monthly survival was lowest among pups in the first 3 months 0.91 [95% CI 0.83–0.95]. After this initial pup stage, \( \phi_{mo} \) was consistently high among juveniles 0.96 [0.93–0.98], second year subadults 0.97 [0.95–0.99], and third year adults 0.98 [0.94–1.0]. Monthly survival decreased among fourth year adults 0.94 [0.80–0.98] and was similar to \( \phi_{mo} \) among adults of unknown age 0.93 [0.92–0.95]. Monthly survival varied among the three sites, but with largely overlapping confidence intervals (Fig. 2). Mean \( \phi_{mo} \) across age classes was highest at Finca (0.97 [0.95–0.98]) and essentially equivalent at Ureña and Esquinas (≈ 0.93 [0.91–0.95]). The overall mean \( \phi_{mo} \) estimate across all site and age classes was 0.95 [0.94–0.95].
Figure 2. Estimates of apparent monthly survival rate $\phi_m$ for *T. tricolor* according to age class, as taken from the best-fit model (model 14, Table 3). Bars indicate the 95% confidence interval. Pup equals first three months of life, Juv equals remainder of first year, Adult refers to individuals of unknown age that were reproductively mature on first capture. Finca (●), Ureña (○), Esquinas (△).

The typical survival curve of mammals generally is a three-stage process, including high mortality among young-of-the-year, lower mortality among adults, followed by an increase in mortality at the onset of senescence (Caughley 1966; Gaillard et al. 1993; Jorgenson et al. 1997). We simplified the age-structure inferred from the best-fit model to a three age class model (the Caughley-like model) for the purpose of estimating representative annual survival rates for this species. We constrained survival to be constant across the three sites and reduced the age-structure to three age classes. The first combined pups and juveniles into a single first-year age class (juveniles). This has the effect of making our juvenile estimates comparable to studies which only
Table 4. Estimates [95% CI] of apparent monthly survival rate $\phi_{in0}$ for *T. tricolor* by age class and site based on the best-fit model (model 14, Table 3). $N =$ number of marked individuals entered into the analysis.

<table>
<thead>
<tr>
<th>Site (N)</th>
<th>Pup [95% CI]</th>
<th>Juvenile [95% CI]</th>
<th>Year 2 [95% CI]</th>
<th>Year 3 [95% CI]</th>
<th>Year 4 [95% CI]</th>
<th>Adult [95% CI]</th>
<th>Across all age classes [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finca (66)</td>
<td>0.93 [0.86–0.97]</td>
<td>0.97 [0.95–0.99]</td>
<td>0.98 [0.96–0.99]</td>
<td>0.99 [0.95–1.0]</td>
<td>0.96 [0.86–0.99]</td>
<td>0.96 [0.94–0.97]</td>
<td>0.97 [0.95–0.98]</td>
</tr>
<tr>
<td>Ureña (65)</td>
<td>0.89 [0.76–0.96]</td>
<td>0.96 [0.91–0.98]</td>
<td>0.97 [0.94–0.99]</td>
<td>0.98 [0.90–1.0]</td>
<td>0.94 [0.78–0.98]</td>
<td>0.92 [0.88–0.94]</td>
<td>0.93 [0.91–0.95]</td>
</tr>
<tr>
<td>Esquinas (114)</td>
<td>0.86 [0.71–0.94]</td>
<td>0.95 [0.89–0.97]</td>
<td>0.96 [0.92–0.98]</td>
<td>0.98 [0.90–0.99]</td>
<td>0.91 [0.73–0.98]</td>
<td>0.91 [0.89–0.93]</td>
<td>0.93 [0.91–0.94]</td>
</tr>
<tr>
<td>Across all sites (245)</td>
<td>0.91 [0.83–0.95]</td>
<td>0.96 [0.93–0.98]</td>
<td>0.97 [0.95–0.99]</td>
<td>0.98 [0.94–1.0]</td>
<td>0.94 [0.80–0.98]</td>
<td>0.93 [0.92–0.95]</td>
<td>0.95 [0.94–0.95]</td>
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</table>
distinguish between young-of-the-year and adults. The second combined 2 and 3 year olds based on similarly high levels of survival (prime-age adults). Lastly, we combined bats ≥ 4 years old and adults of unknown age, again based on similar survival rates (senescent adults). Estimates were made on an annual time scale, allowing for clear inference regarding survivorship and providing rates suitable for use in future study of this species (e.g., population growth models, demographic estimates of effective population size, etc.). Based on the Caughley-like model, the annual apparent survival rate (now \( \phi \)) for juveniles during 2006–2010 was 0.55 (95% CI = 0.41–0.68; Fig. 3) compared to the prime-age adult rate of 0.77 (0.62–0.87), and decreased significantly among senescent adults to 0.45 (0.38–0.52).

![Graph](image)

**Figure 3.** Variance in apparent annual survival rate \( \phi \) estimates according to the Caughley-like model for *T. tricolor*; survival parameters represent an average across sites. Bars indicate the 95% confidence intervals. Juvenile equals young-of-the-year, Prime equals 2 to 3 year old adults, Senescent equals ≥ 4 year olds and bats first captured as adults.
Mean lifespan

We used the estimates from the Caughley-like model to calculate expected mean lifespan for *T. tricolor*. Substituting the juvenile estimate of 0.55 for $\phi_1$ and the prime-age adult estimate of 0.77 for $\phi_A$ in the equation of Brownie et al. (1985; see methods), the MLS of *T. tricolor* was 2.81 years for both sexes (95% confidence interval: 2.08–3.53 years). Thus, a newborn female *T. tricolor* will reproduce 1–2 times, provided that it starts reproducing in the fall of its second year.

Discussion

We modeled variance in apparent monthly survival of *T. tricolor* based on capture-recapture data collected from three local populations. This approach allowed us to identify patterns of mortality resulting from different ecological factors and key life-stages. The results suggest that survival follows an age-structure with three age classes and does not differ between sexes. Survival varied among the three sites, however, survival was not linked with any specific time of year, either seasonal or reproductive. The most parsimonious model suggested that recapture rate varied among capture occasions, and this temporal effect differed between sites.

Population effects

Monthly survival estimates differed spatially, with estimates of survival for each population having broadly overlapping confidence intervals for all age classes (Fig. 2). However, survival probabilities of all age classes varied in parallel across sites, indicating that these variations were caused by site-specific factors to which all ages were similarly sensitive. Differences in mean survival (i.e. across all age classes) between Finca ($\phi_{mo} =$
and Ureña and Esquinas (0.93 [0.91–0.95] for both) were statistically significant (Table 4). Slight differences in monthly survival rate result in large differences when extrapolated to an annual time scale (i.e. Φ_{12}^{mo} = 0.69 for Finca, 0.41 for Ureña and Esquinas). Thus, local conditions can result in potentially large differences in annual survival among _T. tricolor_ populations. Inter-population variability in survival has been detected and emphasized in a number of studies (e.g., Frederiksen et al. 2005; Gaillard et al. 1997; Ozgul et al. 2006), and to date few studies on bats have used the approach of measuring vital rates from multiple populations (Papadatou et al. 2011; Pryde et al. 2006; Pryde et al. 2005). This study supports a growing body of research indicating vital rates can be population-specific, and the general validity of single-population estimates should be treated with caution due to potential bias.

The observed differences in survival are likely due to variation in population-specific characteristics, such as quality of roosting and foraging habitat, parasite or disease prevalence, predation, or age distribution. In some large long-lived mammals, increased population density can affect population age structure, leading to a higher proportion of senescent females with a lower probability of survival than prime-aged individuals (Festa-Bianchet et al. 2003). However, data on density effects on the age structure of bat colonies, as well as senescence effects on bat survival are lacking. Because our ultimate aim was to investigate which of many ecological factors might be affecting monthly survival within the annual cycle of _T. tricolor_, and not to identify the specific causes of inter-population variations _per se_, further studies at the multi-population level should be conducted in order to test specific hypotheses regarding these differences.
Age-structure

According to the best-fitting model, there was significant variation in survival with age (Fig 2). With the exception of a 3 month period of postnatal development, high survival was associated with younger age. Survival was lowest among pups during the first three months of life, at which point it increased with only a marginal differences between juveniles and non-reproductive and reproductive adults, before decreasing significantly at approximately four years of age. This pattern is suggestive of the three stage age-structure common to many mammals (Caughley 1966). Despite differences in local conditions, the pattern of age-structured survival was constant across sites, suggesting age effects on survival are strong within this species.

First-year survival has been found to be consistently lower than adult survival in bats (see Table 5). Factors contributing to reduced survival in young bats likely vary based on stage of development and level of independence. For pre-volant pups, amount of maternal care and investment are important for survival, as well as natal condition and location (Kunz and Hood 2000). Among newly-volant juveniles, risks associated with being an inexperienced flyer may negatively affect survival by increasing vulnerability to predation and reducing foraging efficiency (Pryde et al. 2005; Tuttle and Stevenson 1982). Data on the relative importance of each of these life stages to overall first-year survival are scarce. Estimates of mortality during the pre-weaning period, which includes pre-volancy and early volancy for young bats, are available for only a few vespertilionid species and range from 4% to 11.8% (Foster et al. 1978). Our monthly survival estimates for this period suggest that mortality is much higher in *T. tricolor* (closer to 30%). Support from the data for a model constraining reduced pup survival to 3 months (i.e.
Table 5. Comparison of published bat survival rates from studies where survival was estimated using Cormack-Jolly-Seber methods.

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Survival</th>
<th>Survival</th>
<th>Survival</th>
<th>Survival</th>
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<tr>
<td></td>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
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<td></td>
<td></td>
<td>Juveniles</td>
<td>Adults</td>
<td>Juveniles</td>
<td>Adults</td>
</tr>
<tr>
<td>This study</td>
<td><em>Thyroptera tricolor</em></td>
<td>0.55 [0.41–0.68]</td>
<td>0.77 [0.62–0.87]</td>
<td>0.55 [0.41–0.68]</td>
<td>0.77 [0.62–0.87]</td>
</tr>
<tr>
<td>Keen and Hitchcock</td>
<td><em>Myotis lucifugus</em></td>
<td>0.71 ±0.02</td>
<td>0.82 ± 0.01</td>
<td>0.71 ±0.02</td>
<td>0.82 ± 0.01</td>
</tr>
<tr>
<td>Hitchcock et al. 1980</td>
<td><em>Myotis leibii</em></td>
<td>0.42 ± 0.07</td>
<td>0.76 ± 0.11</td>
<td>0.42 ± 0.07</td>
<td>0.76 ± 0.11</td>
</tr>
<tr>
<td>Hitchcock et al. 1984</td>
<td><em>Eptesicus fuscus</em></td>
<td>0.70 ± 0.06</td>
<td>0.47 ± 0.06</td>
<td>0.70 ± 0.06</td>
<td>0.47 ± 0.06</td>
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<tr>
<td>Hoyle et al. 2001</td>
<td><em>Macronectria gigas</em></td>
<td>0.35–0.46</td>
<td>0.57–0.77</td>
<td>0.29–0.42</td>
<td>0.43–0.66</td>
</tr>
<tr>
<td>Sendor and Simon 2003</td>
<td><em>Pipistrellus pipistrellus</em></td>
<td>0.53 ± 0.10</td>
<td>0.80 ± 0.05</td>
<td>0.53 ± 0.10</td>
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<td>Pryde et al. 2005</td>
<td><em>Chalinolobus tuberculatus</em></td>
<td>0.47–0.72</td>
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<td>0.52–0.83</td>
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<td>Pryde et al. 2006</td>
<td><em>Chalinolobus tuberculatus</em></td>
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<td>Frick et al. 2007</td>
<td><em>Myotis yumanensis</em></td>
<td>0.60–0.80</td>
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<td>0.73–0.89</td>
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<tr>
<td>Schaub et al. 2007</td>
<td><em>Rhinolophus ferrumequinum</em></td>
<td>0.49 ± 0.06</td>
<td>0.91 ± 0.02</td>
<td>0.49 ± 0.06</td>
<td>0.91 ± 0.02</td>
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<tr>
<td>Papadatou et al. 2009</td>
<td><em>Myotis capaccini</em></td>
<td>0.45 ± 0.04</td>
<td>0.86–0.94</td>
<td>0.45 ± 0.04</td>
<td>0.86–0.94</td>
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<tr>
<td>Schorch et al. 2009</td>
<td><em>Nyctalus leisleri</em></td>
<td>0.45 ± 0.04</td>
<td>0.76 ± 0.04</td>
<td>0.45 ± 0.04</td>
<td>0.76 ± 0.04</td>
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<td>Frick et al. 2010</td>
<td><em>Myotis lucifugus</em></td>
<td>0.23–0.46</td>
<td>0.63–0.90</td>
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<td>O'Shea et al. 2010, 2011</td>
<td><em>Eptesicus fuscus</em></td>
<td>0.67 [0.61–0.73]</td>
<td>0.79 [0.77–0.81]</td>
<td>0.67 [0.61–0.73]</td>
<td>0.79 [0.77–0.81]</td>
</tr>
<tr>
<td>Papadatou et al. 2011</td>
<td><em>Eptesicus isabellinus</em></td>
<td>0.72 [0.57–0.93]</td>
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</table>

* No difference detected between sexes.
pre-volant) as opposed to 6 months suggests strong influence of the first few months on first year survival. Our results suggest that, at least in *T. tricolor* first-year survival depends on factors affecting pups prior to independence, rather than factors associated with inexperience (i.e. learning to forage and find roosts). In fact, survival rates among volant juveniles similar to first and second year adults would suggest relatively little survival cost associated with the transition to independence, possibly due to the strong social structure present in this species.

In terrestrial mammals, survival has been shown to be affected by senescence, where the mortality of adult individuals increases after a certain age (Caughley 1966; Descamps et al. 2008; Festa-Bianchet et al. 2003; Loison et al. 1999; Promislow 1991). We would expect the same for bats, and this appears to be the case in the *T. tricolor*. For both sexes in all populations we found clear evidence of senescence. Promislow (1991) demonstrated that among many long-lived mammals, mortality increased immediately after first reproduction and then again for older age classes. This is not the pattern we observe, as the decrease in survival at 4 years comes well after sexual maturity in both sexes. Complex adult age-structure, with the presence of a senescing age class, has not been detected in previous studies of bats (Schorcht et al. 2009; Sendor and Simon 2003), and published information on the effects of senescence on bat population dynamics is lacking. To our knowledge, this is the first study to demonstrate such complex age-structure in any bat species. Our results suggest *T. tricolor* have type 1 survivorship (i.e. late loss) in conjunction with significant first-year mortality. Future analyses of this, and potentially other bat species, which fail to account for complex age-structure and pool
individuals from different age classes may lead to biased estimates of vital rates (Festa-Bianchet et al. 2003).

Senescence has been attributed to the cumulative effects of reproductive activities and to deleterious mutations having their effects later in life (Rose 1991). Increased tooth wear may lower feeding efficiency among bats, yet there is no evidence of a decrease in body mass at older ages (Buchalski, unpublished data). All of these explanations predict an accelerating increase in mortality with age. Based on the length of our study and the apparent age of onset of senescence, it is not possible to determine if bat survival progressively continues to decline with age beyond 4 years. Future research should investigate the extent of longevity in *T. tricolor*, as well as the factors explaining individual differences in survivorship.

*Sex effects*

Most modern capture-recapture studies of bats have found lower annual survival among males than for females (see Table 5), though some found no difference (Schaub et al. 2007; Sendor and Simon 2003). Lower male survival is often due to sex-biased natal dispersal (Boyd and Stebbings 1989; Hoyle et al. 2001; Pryde et al. 2005) and the fact that survival estimates are biased downwards by permanent emigration. Low male survival can also be attributed to polygynous mating systems in which males must compete for access to females, imparting energetic costs and promoting risky behavior (Gerell and Lundberg 1990; Schorcht et al. 2009). Our results suggest little importance of sex differences in *T. tricolor* survival. These results were unexpected given (1) sex differences in the age of first reproduction, and (2) the long reproductive period of both sexes and exceptionally large investment made by females (~ 8 months). The lack of
difference could be due to the pattern of all-sex philopatry observed in *T. tricolor*, i.e. no sex-biased dispersal as is common in many other bat species (see Kerth et al. 2002). Sex differences in survival has been attributed to sexual dimorphism in body size in other mammalian taxa (Promislow 1992), with individuals of the larger sex (typically males) having greater energy requirements and greater mortality during periods of resource shortages. However, size dimorphism in bats is minimal when compared to other mammalian taxa (Williams and Findley 1979). Our results suggest that mortality is mainly due to factors independent of sex or reproduction.

*Annual survival rates, mean lifespan, and senescence*

Our estimated survival rate for juvenile *T. tricolor* (0.55 [0.41–0.68]) was comparable or higher than values reported from several studies of temperate zone bats using CJS models (Table 5). Similarly, prime-age adult survival estimates (0.77 [0.62–0.87]) fall within the range estimated for several temperate bats. Among bats, different life histories are likely to exist along the slow-fast continuum, with short-lived species that are relatively more productive to relatively longer-lived species that are less productive (Bielby et al. 2007). Previous studies of bat longevity indicate tropical species have shorter lives than temperate bat species (see Barclay and Harder 2003; Wilkinson and South 2002). We present evidence that *T. tricolor* is at the long-lived end of this continuum for tropical species, possibly making its survival and life history similar to temperate species. Basic demographic information of other tropical bat species must be known to make strong conclusions.

Annual survival rate estimates from the Caughley-like model allowed us to calculate mean lifespan while accounting for age structure. To date, only three studies
which have detected age-structured survival in bats have used survival estimates to calculate mean lifespan. Two of those (O'Shea et al. 2011; Sendor and Simon 2003) did not use an age-structured model of MLS. Failure to account for reduced juvenile survival has the effect of inflating mean lifespan estimates. For example, substituting our value of prime-age adult survival for *T. tricolor* into a single age class model of MLS (Seber 1973),

\[
\text{MLS} = \frac{1}{-\ln(\phi)}
\]

results in a higher estimate than obtained with full age structure (3.83 vs. 2.81 years). The MLS estimate calculated for *T. tricolor* using both juvenile and prime-age adult survival probabilities were comparable to the only other estimate for bats which accounted for age structure. Schortcht et al. (2009) generated an estimate of 2.26 [1.74–2.78 years] for the temperate species *Nyctalus leisleri*. Although previous studies of mammals have demonstrated the importance adult survival has on influencing population growth rates (Eberhardt 2002), our results suggest caution when using only the adult survival rate, and a model which does not account for age class variation, for estimating MLS. Our findings also support the general conclusion of population ecologists that maximum longevity records are not reliable statistics for understanding demographic processes (Krementz et al. 1989), and that life expectancies based on survival rate estimates are typically much shorter than longevity records (O'Shea et al. 2011).
Conclusion

Though there is a growing literature on vital rate estimation for temperate bats, this study represents the first application of modern capture-recapture modeling methods to estimate survival rate in a neotropical species, taxa which have traditionally gone understudied. Our data, collected over the course of five years from three populations, indicated evidence for site effects on survival, reinforcing the importance of accounting for inter-population variation when estimating representative values of survival for a species. *T. tricolor* show the most complicated age structure described to date for a bat, conforming to a three age class structure common to many species of mammals. Age structure from the best-fit model suggests that the majority of first-year mortality occurs among pre-volant pups, and that survival among volant juveniles rivals that of prime-age adults. Ours is the first modern capture-recapture study to identify a senescent life stage among bats, with survival decreasing significantly well after the age of sexual maturity. Survival rates among juveniles and adults were comparable to several species of temperate bats, suggesting that *T. tricolor* do in fact occupy the slow lane of the slow-fast continuum of life histories among bat species.

Our findings have implications for conservation of bat populations. The negative population trends and deteriorating conservation status of many tropical bat species dictates a need for accurate vital rate estimation. Assessing the impact of anthropogenic modifications to modern landscapes will require estimates of vital rates at multiple populations simultaneously. Therefore, modern capture-recapture models should become an essential tool of the modern bat conservation biologist. We hope that approaches
similar to those we used with this common neotropical species will be considered in future studies of other bat species to advance understanding of their population dynamics.
References


CHAPTER II

WHEN GENES MOVE FARThER THAN OFFSPRING: GENE FLOW BY MALE GAMETE DISPERSAL IN THE HIGHLY PHILOPATRIC BAT SPECIES THYROPTERA TRICOLOR

Introduction

Behavioral patterns of natal dispersal, social group formation based on kinship, and mate choice have been shown to directly shape population genetic structure in many mammalian species (Chepko-Sade and Halpin 1987; Holekamp et al. 2012; Storz 1999). These factors interact, as dispersal is often associated with kin competition and inbreeding avoidance (Costello et al. 2008; Moore and Ali 1984; Pusey 1987), and mate choice has been shown to be influenced by levels of social association and genetic relatedness (Cohas et al. 2008; Pusey and Wolf 1996). An understanding of all three processes, and their interactions, is therefore requisite to understanding the forces that structure populations of social mammals.

Natal dispersal has long been proposed as an adaptation for close inbreeding avoidance (Greenwood 1980; Johnson and Gaines 1990) and the primary mechanism of gene flow between populations of organisms (Wright 1931), yet some species are characterized by extreme philopatry. In these situations offspring of both sexes continue to reside on or near the natal territory beyond the age of reproductive maturity (e.g., among mammals; Amos et al. 1993; Blumstein and Armitage 1999; Clutton-Brock et al. 2001; Peacock 1997; SilleroZubiri et al. 1996; Stockley et al. 1993; Waser and Jones 1993).
1983). For species that maintain solitary territories, this results in high relatedness among nearest neighbors (Peacock and Smith 1997; Winters and Waser 2003). In social (i.e. gregarious) species, the result is group formation based on kinship (Greenwood 1980). Extreme philopatry also occurs in groups of non-cooperatively breeding mammals when fitness benefits are accrued by associating with close kin (Amos et al. 1993), or when ecological or morphological constraints limit dispersal (Burland et al. 1999; Miller-Butterworth et al. 2003).

Highly philopatric species typically display low rates of inbreeding due to various mating behaviors, including mate choice based on relatedness (Dobson et al. 1997; Hoogland 1982; Peacock and Smith 1997), extra-pair copulations (Fietz et al. 2000; Goossens et al. 1998; Roemer et al. 2001), and mating forays (i.e. temporary movement outside the typical home range or social group for the purpose of finding a mate) (Amos et al. 1993; Winters and Waser 2003). In the context of gene flow, mating forays distribute male gametes between social groups, or over greater geographic distances than young disperse. This phenomenon has been referred to as “gamete dispersal” (Waser and Elliott 1991) creating patterns of gene flow analogous to plants in which pollen dispersal occurs over large areas and seed dispersal is limited (Crawford 1984).

Spix’s disk-winged bat (*Thyroptera tricolor*) is a gregarious species that displays all-offspring philopatry, forming social groups consisting of one or more reproductive females and offspring of both sexes from multiple years (Chaverri and Kunz 2011a). This bat is morphologically specialized to roost within the furled, developing leaves of *Heliconia* and *Calathea* plants (Wilson and Findley 1977). Groups exhibit fidelity to small roosting home ranges encompassing one or more patches of plants that continually
produce new leaves (Chaverri and Kunz 2011b; Vonhof et al. 2004). Group cohesion is maintained through highly specific vocalizations between members during the location of roosts (Chaverri and Gillam 2010). Given such extreme natal group philopatry and highly limited spatial movement among both sexes, we sought to investigate the effect of such a life history on population genetic structure, and determine whether behaviors associated with mate choice reduce the potential for close inbreeding and facilitate gene flow.

For gregarious, highly philopatric species, the implications of mate choice should be assessed within the context of both the social organization, and spatial genetic structure of the breeding population. Parentage analysis and relatedness estimates, performed in conjunction with capture-recapture surveys, can describe the spatial distribution, genetic relationships, and social interactions between mated pairs and their offspring (Vignieri 2007; Winters and Waser 2003). Genetic spatial autocorrelation analysis (Epperson and Li 1996) is highly effective for detecting detailed patterns of spatial genetic structure in animals (Peakall et al. 2003). A useful model for estimating the effect of dispersal (gamete or natal) on gene flow is that of isolation-by-distance, IBD (Wright 1943). Populations are described as collections of panmictic groups (i.e. genetic neighborhoods), and the scale of gene flow, or neighborhood area, can be estimated directly from observed dispersal distances. An IBD modeling approach also allows for indirect, genetic estimates of gene flow (Rousset 1997), as geographically closer individuals are anticipated to be more similar genetically. Comparisons of direct and indirect estimators have been successfully applied to studies of movement and gene flow in other mammalian species (Broquet et al. 2006; Rousset 2000; Selonen et al. 2010),
with the benefit of addressing potential biases associated with direct estimates of dispersal from field data.

Here we investigate patterns of mate selection and resultant gene flow in a highly philopatric, gregarious bat. Due to this species’ strong social group cohesion and philopatry to small roosting home ranges, we chose to evaluate mate choice both in a social and spatial (population-level genetic structure) context. We follow a behavioral-to-genetic level approach by using a combination of capture–recapture and microsatellite data to identify mated pairs and their genetic, social, and spatial relationships. We combine home range data with individual-, group-, and population-level genetic analyses to: (i) estimate relatedness within social groups; (ii) determine whether positive spatial genetic structure occurs at the individual or group level; (iii) evaluate patterns of mate choice in relation to geographic distance and levels of relatedness; (iv) evaluate the collective evidence for mate choice behavior to reduce the incidence of close inbreeding and facilitate gene flow; and (v) infer the spatial scale of gene flow, by comparing direct (demographic) and indirect (genetic) estimates of gamete dispersal distances within an isolation-by-distance model context. Our results suggest that mating forays represent a behavioral adaptation for close inbreeding avoidance in *T. tricolor* and that gamete dispersal facilitates gene flow in this species, in lieu of natal dispersal of young.

Methods

*Study species*

Spix’s disk-winged bat is a small (3–4 g) insectivorous species found in neotropical forests from central Mexico to southern Brazil (Wilson and Findley 1977). Social groups, or individuals that share the same roost, are comprised of 2 to 14
individuals, and can range from entirely male to entirely female. Females give birth to one pup per year throughout their lives (i.e. seasonal monoestry; Chaverri and Vonhof 2011), while patterns of male reproductive success are currently unknown. Males reach sexual maturity within their first year, whereas females are characterized by a second developmental (i.e. subadult) year. Young-of-the-year of both sexes, and subadult females can be readily distinguished from adults, as pelage color changes from dark gray to light brown within the first year and sexual characteristics become conspicuous (e.g., descended testicles, keratinized nipples, lactation, pregnancy, etc.). There is no evidence for cooperative breeding in *T. tricolor*.

*Field methods and study populations*

This study was conducted at two sites within southwestern Costa Rica (Fig. 4). The Km23 site (8°38'N, 83°05'W; 93.6 ha), is located within a matrix of primary and secondary wet tropical forest, and agricultural lands, with abundant *Heliconia imbricata* and *Calathea lutea* present in the understory. The Sirena site (8°28'N, 83°35'W; 104.0 ha) is located within Corcovado National Park and is composed of continuous stands of primary and late secondary forests, with *Heliconia* spp. patches scattered in the understory.

Field surveys were conducted from 2008 through 2011 within pre-identified patches of habitat, every 2 to 4 weeks between the months of May to September. Groups were captured at the roost, and bats were fitted with individually numbered metal wing bands (Porzana Ltd.), sexed, aged, and reproductive condition assessed. Age was classified as juvenile (young-of-the-year), subadult (females only), and adult. A 3 mm biopsy punch of skin tissue was taken from each wing and stored in 5 M NaCl with 20%
dimethyl-sulfoxide solution for use in genetic analyses. All capture locations were recorded on a hand-held GPS unit.

Figure 4. Location of two Spix’s disk-winged bat (Thryroptera tricolor) local populations in southwestern Costa Rica where data were collected from 2008 to 2011.

We captured 768 individuals from 115 social groups among the two sites. This sample included 379 bats in 48 groups from Km23, and 389 bats in 67 groups from Sirena. Social groups were defined using Newman’s (2004) modularity modified for weighted networks within the program SOCPROG (Whitehead 2009), with modularity values of 0.95 and 0.94 for Km23 and Sirena, respectively. Modularity has an expected value of 0.0 for randomly assigned groups and equals 1.0 if there is no association between members of different groups. Groups were typically of mixed sex, ranging from 2 to 20 bats (mean = 6.8, mode = 5). Sex ratios [M/(M + F)] for the two sites were not significantly different from parity (Km23 = 0.54 [χ² = 2.22, P = 0.136], Sirena = 0.48 [χ²
Of our total captures, 279 bats (139 from Km23 and 140 from Sirena) were first sampled as juveniles or female subadults and were thus of known age.

Roosting home ranges were constructed for all individuals captured three or more times, using a 100% minimum convex polygon (MCP) in ArcGIS 10.0 (ESRI Redlands, CA). The centroid for each home range was used in all pairwise comparisons of geographic distance. Mean roosting home range size for both sites (~ 0.20 ha) was similar to previously documented estimates for this species (0.19 ha in Vonhof et al. 2004; 0.14 ha in Chaverri and Kunz 2011), with no difference in the size of male and female home ranges (t-test: $t_{189} = 1.97, P = 0.767$). For individuals with fewer than three captures, either single capture locations, or the center point between two capture locations were used for distance comparisons. Group roosting home ranges were constructed using the 100% MCP of the combined capture locations for all individual group members (see above). The centroid for this polygon was used in all pairwise distance comparisons between groups.

**Molecular analysis**

DNA was extracted from wing tissue using Qiagen® DNeasy Blood & Tissue Kits. All samples were genotyped at nine polymorphic microsatellite loci (Table 6) developed by Vonhof et al. (2001). Polymerase chain reaction amplifications were performed on a PTC–200 Peltier Thermal Cycler (MJ Research) and microsatellite fragment length was resolved on an ABI 3730 capillary electrophoresis system with an internal size standard and scored using GeneMarker 1.9 (Softgenetics®).

Allele frequencies, and observed and expected heterozygosities, were calculated for each locus. We tested deviations from Hardy–Weinberg equilibrium in GENEPOP 4.2
(Raymond and Rousset 1995) using the exact probability test, with Markov chain parameters set to 100 batches with 1,000 iterations per batch. Nei’s estimator of $F_{IS}$ (1987) was calculated across all loci for each population, and within each social group, using FSTAT 2.9.3 (Goudet 2001). The number of alleles per locus, observed and expected heterozygosities, the probability of excluding an incorrect parent when no parents are known (Jamieson and Taylor 1997), and $F_{IS}$ values are shown in Table 6. We confirmed the presence of a null allele at locus Tt13 using the program ML-RELATE (Kalinowski et al. 2006), estimated its frequency at 0.05, and adjusted our calculations of parentage and relatedness accordingly (see below).

Table 6. Genetic diversity and exclusionary power of nine microsatellite loci for Spix’s disk-winged bat (Thyroptera tricolor) within two populations expressed as (N) the number of alleles ($H_O$) observed and ($H_E$) expected heterozygosities per locus, ($F_{IS}$) the inbreeding coefficient, and the probability of excluding an incorrect parent when no parents are known ($P_{E-1st}$). Mean within- social group $F_{IS}$ estimates are shown below population totals. Deviation from Hardy-Weinberg equilibrium in a single locus indicated by **$P \leq 0.01$, ***$P \leq 0.001$.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Km23</th>
<th>Sirena</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>$H_O$</td>
</tr>
<tr>
<td>Tt2</td>
<td>16</td>
<td>0.863</td>
</tr>
<tr>
<td>Tt5B</td>
<td>8</td>
<td>0.765</td>
</tr>
<tr>
<td>Tt8</td>
<td>19</td>
<td>0.902</td>
</tr>
<tr>
<td>Tt10</td>
<td>15</td>
<td>0.900***</td>
</tr>
<tr>
<td>Tt13</td>
<td>15†</td>
<td>0.789**</td>
</tr>
<tr>
<td>Tt30</td>
<td>18</td>
<td>0.942</td>
</tr>
<tr>
<td>Tt33</td>
<td>17</td>
<td>0.858</td>
</tr>
<tr>
<td>Tt34</td>
<td>5</td>
<td>0.517</td>
</tr>
<tr>
<td>Tt37</td>
<td>21</td>
<td>0.821**</td>
</tr>
<tr>
<td>B</td>
<td>Overall</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Within-group</td>
<td>-0.117</td>
</tr>
</tbody>
</table>

†Number of alleles includes a null allele identified during analysis.
Population structure

We performed analysis of spatial autocorrelation using the genetic distance-based, multiallele, multilocus approach developed by Smouse and Peakall (1999). Geographic and genetic pairwise distance matrices were calculated in GenAlEx 6.5 (Peakall and Smouse 2012). The 95% confidence interval around the genetic autocorrelation coefficient, $r_c$, estimate for each distance class was estimated via bootstrapping as described in Peakall et al. (2003). We also compared the patterns of spatial genetic structure between males and females by generating separate geographic and genetic distance matrices for each sex. To examine genetic similarity between social groups within the context of potential mate choice, we used GenAlEx to perform spatial autocorrelation analysis at the group level. A matrix of the mean genetic distances across all pairs of individuals representing a specific among group contrast, and a matrix of geographic distances among group home range centroids (described above) were used as the input for group-level spatial autocorrelation analysis. Selection of distance classes for all autocorrelation analyses followed the methodology of Beck et al. (2008).

Parentage analysis

Parents were assigned to individuals of known age (i.e. juveniles and subadult females) using PARENTE (Cercueil et al. 2002). For our candidate parent data sets we included all individuals captured within a site over the entire study, and we limited our assignments to individuals of known age to guarantee the directionality of the parent/offspring relationship. The proportion of each population sampled was parameterized based on capture-recapture estimates of population size (see below) and typing error rate was parameterized to 0.01 to account for potential scoring errors. Triad
assignments made with 90% confidence and a maximum of one mismatch per parent were retained for further analyses of mated pairs. When available, field observations of pre-weaning mother-offspring relationships (i.e. pup still attached to mother’s nipple) were used to verify maternal assignment.

Simulation studies have demonstrated that null alleles with a frequency of 0.2 or less introduce inconsequential bias during molecular parentage analysis by underestimating the exclusion probability (Dakin and Avise 2004). The estimated frequency of the null allele at locus Tt13 was 0.05. The exclusion probability for this locus was 0.935, while the overall exclusion probability of all markers combined was 1.0 (Table 6). We therefore felt we were not assigning parents with a false level of confidence. To avoid falsely excluding parents due to the null allele, we required that exclusions be based on mismatches at more than one locus. Running the analysis twice, adjusting the number of allowable mismatching loci from one to two, resulted in identical assignments across the two analyses.

**Relatedness**

Maximum-likelihood estimates of pairwise relatedness, $R$, among all individuals within each population were calculated using ML-RELATE, while accounting for the presence of a null allele at locus Tt13. These estimates were imported into GenAlEx and mean pairwise relatedness for each roosting group (n = 101) was calculated. Bootstrap resampling of individuals within groups (1,000 permutations) was used to generate 95% confidence intervals around relatedness estimates. To determine if non-random mating was occurring based on relatedness preferences, a Monte Carlo resampling scheme with 10,000 iterations was created using R 2.15 (R Development Core Team 2012). In each
iteration, \( R \) values were selected randomly from a matrix comparing all adult males with all adult females. The number of simulated “matings” drawn in each iteration was equal to the number of mated pairs identified at each site during the study, and mean \( R \) was calculated for each iteration to estimate the 95% confidence limits of possible relatedness. We wished to confirm previous observations of all-offspring philopatry in *T. tricolor* (Chaverri and Kunz 2011a) using genetic data, testing whether males engage in delayed natal dispersal associated with first breeding. We compared mean pairwise relatedness of fathers identified through parentage analysis to group mates pre- and post-reproduction to determine if reproductive males were truly philopatric and continued to roost with close kin.

**Direct estimate of gamete dispersal distances**

Under all-offspring philopatry, offspring will belong to the same social group as their mother, with gene flow limited to dispersal of male gametes during mating. Patterns of mate choice therefore have important implications for population genetic structure. We estimated gamete dispersal distance, both directly (demographic) and indirectly (genetic), to quantify the effect of mate choice on the spatial extent of gene flow at each site. In the absence of natal dispersal, Wright’s equation for neighborhood area is expressed as

\[
4\pi\sigma^2/2, \text{ where } \sigma^2 \text{ represents the mean square axial parent-offspring dispersal distance, and the } 1/2 \text{ accounts for the fact that gene flow only occurs through the movement of male gametes (Crawford 1984). Therefore, } \sigma^2 \text{ is equivalent to the squared axial distances between fathers and offspring. Assuming symmetrical dispersal in two dimensions, } \sigma^2 = 1/2 \cdot r^2 \text{ where } r \text{ is the Euclidean distance between fathers and offspring (See Appendix 1 in Sumner et al. 2001 for detailed explanation). We substituted the home range distances}
\]
between fathers and offspring (see above) for \( r \), squared and summed these distances for all \( n \) pairs, and calculated \( \sigma^2 = \frac{1}{2n} \Sigma r^2 \) (Waser and Elliott 1991). The resultant model estimate of mean gamete dispersal distance within a population is equivalent to the radius of the calculated neighborhood area, or \( \hat{r} \).

**Indirect estimates of gamete dispersal distance**

Direct methods of estimating neighborhood area draw upon observed father-offspring distances, and are subject to potential sampling biases, primarily due to underrepresentation of long-distance events. This makes indirect genetic-based estimates of dispersal distance particularly useful for comparison. Rousset (1997) demonstrated that genetic neighborhood area can be estimated from the slope of the regression of pairwise genetic distances \( F_{ST}/(1 – F_{ST}) \) on the logarithm of geographical distances, \( d \), between all individuals or groups within a population. The inverse of the slope, \( b \), of the regression line is an estimate of the product \( 4D\pi\sigma^2 \), where \( D \) is the density of breeding adults in the population. Thus, with a density estimate one can calculate genetic neighborhood area indirectly.

We used GENEPOP 4.2 to estimate \( b \) and compute the 95% confidence interval based on an advanced Bayesian computation bootstrapping procedure. To estimate the density of breeding adults we first calculated the area of both study sites in ArcGIS. Population size was then estimated via capture-recapture data analysis in MARK (White and Burnham 1999). The Cormack-Jolly-Seber model based on live recaptures in an open population (Lebreton et al. 1992) was used to estimate survival, \( \phi \), and recapture, \( p \), probability. We then followed the methodology of Hoyle et al. (2001) and estimated the population size \( N_i \) for each capture occasion \( i \), as \( n_i/p_i \), where \( n_i \) is the number of bats.
captured on occasion \( i \) and \( p_i \) is the estimated recapture probability for that occasion. Approximate 95% confidence intervals were calculated as \( \hat{N}_i \pm 2se(N_i) \) where \( se(N_i) \) is the standard error of the estimate given by the equation 
\[
se(N_i) = n(se[p])/p^2
\]
(Lettink and Armstrong 2003). We then calculated a single population size for each site by weighting each estimate by the standard error and calculating the weighted mean. We estimated adult population size by multiplying the above estimate by the proportion of bats captured each year that were reproductive adults. Adult population size was then divided by site area to estimate the density of breeding adults (adult/m\(^2\)).

Results

*Genetic variation, group relatedness and population structure*

Alleles per locus ranged from 5 to 23 resulting in 100% power of parentage exclusion (Table 6). Population \( F_{IS} \) values were low (overall values: 0.010 for Km23; 0.037 for Sirena), and negative within-group \( F_{IS} \) values indicated social groups were outbred (– 0.117 for Km23 and – 0.084 for Sirena). Within-group mean pairwise relatedness estimates ranged from 0.053 to 0.359 (Fig. 5a) with a mean of 0.125 for Km23, and ranged from 0.040 to 0.364 (Fig. 5b) with a mean of 0.153 for Sirena, placing the average level of relationship between group-mates at the tertiary level (e.g., first cousins, avuncular, etc.). Mean pairwise relatedness within groups was significantly greater than zero in 84% (85/101) of all groups.
Figure 5. Mean within-group pairwise relatedness estimates for both (a) Km23 and (b) Sirena organized by group size. Error bars represent bootstrapped confidence intervals around the mean. The reference line represents average relatedness within groups across each site.

Spatial autocorrelation analysis confirmed significant positive spatial genetic structure among individuals over short distances (group-mates), but not among groups (Fig. 6) for both sites. For individuals (Fig. 6a), the first 2-3 distance classes represent within-group comparisons due to imperfect home range overlap (i.e. not all group members were captured every survey occasion). Significant positive structure is present among individuals at distances of less than 100 m. The spatial extent of genetic correlation was the same for both sexes, with both sexes closely matching the outcomes for the total data set (Fig. 7). However, the strength of correlation among females was significantly greater than that of males ($r_c = 0.135$ vs. $0.364$ between 0 and 25 m for Km23; $r_c = 0.148$ vs. 0.264 for Sirena, 95% CIs not overlapping). Group level spatial
analysis revealed no significant local positive autocorrelation (Fig. 6b). The combined relatedness and genetic spatial autocorrelation analyses suggest that 1) bats within social groups are frequently related, and 2) are generally unrelated to bats in neighboring groups. This suggests that mating options within social groups are largely limited to close kin, whereas extra-group mating, even with nearest-neighbors, poses no risk of inbreeding.

Figure 6. Correlogram plots of the spatial autocorrelation coefficient $r_c$ as a function of distance at both sites for (a) individuals and (b) groups. Upper and lower 95% error bars about $r_c$ as determined by bootstrap resampling are shown.
Figure 7. Correlogram plots of the spatial autocorrelation coefficient $r_c$ as a function of distance for individuals by sex and site. Upper and lower 95% error bars about $r_c$ as determined by bootstrap resampling are shown.

**Parentage analysis and mated pair identification**

We were able to assign at least one parent to 203 offspring (108 for Km23 and 95 for Sirena), and both parents to 34 offspring (22 for Km23 and 12 for Sirena), identifying as many mated pairs. Relatedness estimates between parent-offspring dyads conformed to the expectation of 50% of alleles identical by descent (mean $R = 0.52$) and genetically-assigned mothers matched field-assigned mothers in 28 out of 29 cases. In the last instance, the mother and offspring shared an allele at every locus, but the high frequencies of the shared alleles within the population resulted in an assignment probability below threshold. All mother-offspring dyads belonged to the same social group. In 13 instances fathers were identified but mothers were not, due to unsuccessful capture or low assignment probabilities among genetically compatible mother-offspring dyads. In these cases we used the offspring’s home range as a surrogate for the unidentified mother and compared fathers and their offspring instead. A total of 47 comparisons were made (31 for Km23 and 16 for Sirena) for the purpose of determining group membership and geographic distance between mated pairs.
Mated pair group membership and spatial distribution

No mated pair belonged to the same social group, but in three cases where the mother could not be identified, offspring and fathers were members of the same group. This indicates that mating among group members does potentially occur, but we have no estimate of relatedness between mated individuals in these instances. Mated pairs rarely (3 out of 47) had roosting home ranges that overlapped, with the majority of pairs separated by large distances when compared to average home range size. Mated individuals at Km23 were separated by 42.8 to 1,418.8 m with a mean of 516.0 (median = 480.4; Fig. 8a). Distances between mates at Sirena were similar, ranging between 1.1 and 1,056.7 m with a mean of 499.1 (median = 457.1; Fig. 8b). Mean distance between nearest-neighboring roosting groups was 48.8 m (SE = 4.88) at Km23 and 46.6 m (SE = 4.22) for Sirena (Fig. 8). These results indicate that reproductive individuals selected mates farther away than nearest-neighbors the majority of the time (Kolmogorov-Smirnov test, \( P < 0.001 \) for both sites).

Mated pair relatedness

Mean relatedness between mated pairs was 0.037 [bootstrap 95% CI 0.018–0.058] for Km23 and 0.073 [0.026–0.130] for Sirena (Fig. 9). Mean relatedness between mates did not fall outside the Monte Carlo distribution of possible matings (0.052, [0.021–0.092] for Km23 and 0.052, [0.012–0.107] for Sirena) nor the 95% confidence distribution of the population means (0.052, [0.048–0.057] for Km23 and 0.052, [0.047–0.056] for Sirena), indicating that mates are no more or less related than random. Mean relatedness between mated pairs was significantly lower than the within-group mean pairwise relatedness (Mann–Whitney \( U \)-test, \( P < 0.01 \) for each site; Fig. 9).
Figure 8. Frequency distributions as proportions for the pairwise distances between nearest-neighboring groups (gray) and mated pairs (black) at (a) Km23 and (b) Sirena. The distributions are significantly different for both sites based on Kolmogorov-Smirnov tests ($P < 0.001$ for each). Dashed lines represent direct estimates of mean gamete dispersal distance ($\hat{r}$), while dotted lines represent indirect estimates.
Figure 9. Mean pairwise relatedness, $R$, between mated pairs (mated), simulated random mating based on Monte Carlo resampling of reproductive adults (simulated), all adults sampled at each site (population), and within groups (group) for Km23 (●) and Sirena (○). Bars are bootstrapped 95% confidence intervals based on 10,000 permutations of the data.

**Relatedness of fathers to group mates, pre- and post-reproduction**

We found no significant difference in mean pairwise relatedness of the 47 identified fathers to their group-mates pre- and post-reproduction (Mann–Whitney $U$-test, $P = 0.455$). Relatedness estimates of breeding males to other members of their roosting group pre- (median = 0.233) and post-reproduction (median = 0.193) were comparable to overall estimates of group relatedness (0.141). These results suggest that reproductive male *T. tricolor* continue to roost with close kin after first breeding.

**Gamete dispersal distance and genetic neighborhood area**

Direct estimates of mean axial square dispersal distance $\sigma^2$ based on pairwise father-offspring distances from Km23 resulted in a genetic neighborhood area of $1.19 \cdot 10^6$ m² (119 ha) and mean gamete dispersal distance $\hat{r}$ of 615.1 m (Table 7). Direct
estimates for Sirena were slightly less, with a neighborhood area of $1.02 \cdot 10^6$ m$^2$ (102 ha) and mean gamete dispersal distance of 569.3 m. These values likely represent underestimates due to finite study area sizes and potential inability to detect dispersal events at the upper end of the distribution of distances.

Table 7. Summary for direct demographic and indirect genetic methods of estimating neighborhood area, mean square axial parent-offspring dispersal distance $\sigma^2$, mean gamete dispersal distance $\hat{r}$, and the ratio of indirect/direct estimates of gamete dispersal distance.

<table>
<thead>
<tr>
<th>Site</th>
<th>Km23</th>
<th>Sirena</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Indirect</td>
</tr>
<tr>
<td>$\sigma^2$ (m$^2$)</td>
<td>1.89 $\cdot$ 10$^5$</td>
<td>—</td>
</tr>
<tr>
<td>Neighborhood area (m$^2$)</td>
<td>1.19 $\cdot$ 10$^6$</td>
<td>2.04 $\cdot$ 10$^6$</td>
</tr>
<tr>
<td>$\hat{r}$ (m)</td>
<td>615.1</td>
<td>806.4</td>
</tr>
<tr>
<td>ratio</td>
<td>1.31</td>
<td>2.35</td>
</tr>
</tbody>
</table>

ArcGIS calculations of site area resulted in $9.36 \cdot 10^5$ m$^2$ (93.6 ha) for Km23 and $1.04 \cdot 10^6$ m$^2$ (104 ha) for Sirena (Table 8). Capture-recapture analyses estimated the adult population size for Km23 at $144.2 \pm 23.3$ and Sirena at $151.2 \pm 14.2$, resulting in adult density estimates of $1.54 \cdot 10^{-4}$ and $1.45 \cdot 10^{-4}$ adult/m$^2$, respectively (Table 8). A small but positive slope, $b$, of the regression of $F_{ST}/(1 − F_{ST})$ vs. ln(distance) was observed at both sites (Fig. 10). The slope estimate for Km23 was $3.18 \cdot 10^{-3}$, 95% confidence interval [$-2.29 \cdot 10^{-3} – 5.99 \cdot 10^{-3}$]. For Sirena, $b$ was estimated at $1.22 \cdot 10^{-3}$ [$-3.81 \cdot 10^{-3} – 8.07 \cdot 10^{-3}$]. Mantel test results based on 10,000 permutations indicated that the slopes were marginally significant ($P$–values of 0.065 and 0.104 for Km23 and Sirena, respectively) and lower confidence intervals were negative in both cases, which translates into an infinite dispersal estimate. Such results are not unexpected in populations with localized dispersal, as the expected patterns of isolation by distance are often weak resulting in low
power for the Mantel test to reject the null hypothesis (Rousset 2008). Several studies utilizing the same methodology have found similar patterns of IBD (i.e. small slope, large scatter) and have demonstrated that estimates of \( b \) provide biologically meaningful inference (Broquet et al. 2006; Puebla et al. 2012; Sumner et al. 2001). The indirect estimate of neighborhood area for Km23 was \( 2.04 \cdot 10^6 \text{ m}^2 \) (204 ha), resulting in a mean gamete dispersal distance of 806.4 m. The indirect estimate of neighborhood area for Sirena was \( 5.63 \cdot 10^6 \text{ m}^2 \) (563 ha), resulting in a \( \hat{r} \) value of 1,340 m. Indirect methods resulted in larger estimates of neighborhood area and mean gamete dispersal distance for both sites (Table 8; Fig. 8), with the indirect estimates of \( \hat{r} \) being 1.31 times the direct estimate at Km23 and 2.35 times the direct estimate at Sirena.

Table 8. Isolation by distance (IBD) and density estimates for the two study populations. IBD slope is the slope of the IBD regression with bootstrap confidence intervals, Mantel test \( P \)-value (10,000 permutations), and \( D \) adult density estimate.

<table>
<thead>
<tr>
<th>Site</th>
<th>Km23</th>
<th>Sirena</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD slope (CI)</td>
<td>( 3.18 \cdot 10^{-3} ) (( -2.29 \cdot 10^{-3}, 5.99 \cdot 10^{-3} ))</td>
<td>( 1.22 \cdot 10^{-3} ) (( -3.81 \cdot 10^{-3}, 8.07 \cdot 10^{-3} ))</td>
</tr>
<tr>
<td>( P )-value</td>
<td>0.065</td>
<td>0.104</td>
</tr>
<tr>
<td>Density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study site area (( \text{m}^2 ))</td>
<td>( 9.36 \cdot 10^5 )</td>
<td>( 1.04 \cdot 10^6 )</td>
</tr>
<tr>
<td>( N ) (adults)</td>
<td>144.2 ± 23.3</td>
<td>151.2 ± 14.2</td>
</tr>
<tr>
<td>( D ) (adult/( \text{m}^2 ))</td>
<td>( 1.54 \cdot 10^{-4} ) (( 1.29 \cdot 10^{-4}, 1.79 \cdot 10^{-4} ))</td>
<td>( 1.45 \cdot 10^{-4} ) (( 1.32 \cdot 10^{-4}, 1.59 \cdot 10^{-4} ))</td>
</tr>
</tbody>
</table>

Discussion

*Within-group and population genetic structure*

Estimates of mean group relatedness revealed that the majority of roosting groups sampled were comprised of close kin. The individual-level spatial autocorrelation
analysis exhibited strong positive genetic correlation within the scale of a typical group home range. This is not surprising given that spatial autocorrelation estimates of \( r_c \) and relatedness estimates are strongly correlated (Banks et al. 2005). Females showed stronger within-group structure than males. Though such results have been attributed to sex-biased dispersal (Peakall et al. 2003), we attribute the higher genetic correlation
among females to the matrilineal group structure within *T. tricolor*, which should result in higher female-female relatedness. Both study populations showed nonsignificant genetic correlations among groups over all distance classes, suggesting that matings between nearest-neighbors should pose no risk of close inbreeding. Spatial autocorrelation analyses, the coincidence of mother-offspring home ranges, and high relatedness of reproductive males to group-mates pre- and post-reproductive event all suggest a lack of natal dispersal, further supporting previous observations of all-offspring philopatry in *T. tricolor*. The pattern of population genetic structure we detected appears to reflect the combined absence of natal dispersal, formation of social groups representing distinct lineages unrelated to neighboring groups, and small roosting home ranges occupied by groups.

*Patterns of mate choice*

This study provides evidence that extra-group paternity is common. Successful mating between group members could only be inferred from three instances where father and offspring belonged to the same group, but mothers were not identified. However, high mean heterozygosities and low to negative $F_{IS}$ values suggest that successful mating between close kin is rare. Furthermore, mated pairs rarely (6%) had roosting home ranges that overlapped, and the majority of females successfully mated with non-neighboring males separated by relatively large distances (Fig. 8). Monte Carlo resampling of reproductive adults suggests that mate selection was not based on preferences for intermediate levels of relatedness (i.e. optimal inbreeding) as has been observed in some small mammals (Hoogland 1982; Peacock and Smith 1997), and pairwise relatedness between mated pairs was significantly lower than mean relatedness within groups.
Therefore, mating forays facilitated reproduction between unrelated individuals from different social groups separated by several roosting home ranges.

Distances between mated pairs were significantly greater than necessary to avoid close inbreeding (i.e. the distance between unrelated nearest-neighbors of the opposite sex), suggesting mate choice is not simply based on genetic similarity, but potentially on the spatial distribution of suitable mates. Mate selection based on spatial distribution has rarely been observed (Winters and Waser 2003), but perhaps this is simply due to an inability or failure to look for it. Other species of bats which use leaves as roosting habitat, primarily tent-roosting bats within the family Phyllostomidae, do not display the level of philopatry observed in *T. tricolor*, and mating tends to occur among roosting group members (Kunz and McCracken 1996; McCracken and Wilkinson 2000). The spatial scale over which *T. tricolor* mated pairs are distributed suggests that encounters occur in association with foraging or night roosting. There is currently no information regarding the foraging ecology of *T. tricolor*, and additional work is needed to elucidate the specific behaviors which bring mates together (e.g., movement of one or both sexes, location and timing of mating, etc.). Knowledge of foraging home range size, whether group and non-group members forage together, and the distribution and use of night roosts, would aid in predicting the frequency and spatial scale over which suitable mates encounter one another. It is important to recognize that although mate choice appears to occur at the scale of the foraging home range, the effects of mate choice on population genetic structure are ultimately realized at the scale of the roosting home range. Dispersal of male gametes well beyond the spatial extent of permanent individual movement
maintains gene flow in the absence of natal dispersal and shapes population genetic
structure in *T. tricolor*.

*Gamete dispersal distances and genetic neighborhood size*

Comparisons of direct and indirect estimates of genetic neighborhood area
demonstrate the spatial scale over which male gamete dispersal facilitating gene flow in
*T. tricolor*. The result is a pattern of spatial genetic structure in *T. tricolor* which is quite
unique among mammals, more closely resembling plants with limited seed dispersal
where gene flow is primarily mediated through dispersal of pollen (Crawford 1984).
Despite microsatellite loci providing reasonable power to detect parent-offspring
relationships, and capture-recapture analyses indicating high percentages of each
population recaptured annually (53.4–95.8 %), our limited success with paternal
assignment (~ 12%) suggests that many fathers resided outside the study areas. We
therefore conclude that our direct estimates are biased low and underestimate mean
gamete dispersal distance, $\hat{r}$. This expectation is supported by larger indirect (i.e.
genetic) estimates for both sites (Fig. 8). Direct and indirect estimates for Km23 showed
reasonable agreement, with indirect estimates of $\hat{r}$ being 1.31 times direct estimates
(Table 8). The relatively small discrepancy between direct and indirect estimates for
Km23 suggests that the spatial scale of sampling was nearly appropriate for
characterizing the spatial extent of gene flow, despite the fact that some fathers went
unsampled. If we treat the direct and indirect estimates as upper and lower bounds on the
extent of gene flow, assuming an average home range area of 0.2 ha (see methods), $\hat{r}$
estimates (615.1–806.4 m) suggest mated individuals will be separated by distances
equivalent to 13–18 roosting home ranges on average. Similarly, genetic neighborhood
area estimates \((1.19 \cdot 10^6 - 2.04 \cdot 10^6 \text{ m}^2)\) suggest that the mating system of \(T. \text{ tricolor}\) acts to disperse male gametes across an area representing 595 to 1,020 home range equivalents.

Estimates for Sirena showed less agreement than for Km23, with indirect estimates of \(\hat{r} = 2.35\) times direct estimates. Direct estimates of neighborhood area and \(\hat{r}\) were comparable to Km23, likely due to our attempt to standardize study area size. However, indirect estimates were much larger for Sirena, resulting in a genetic neighborhood area \((5.63 \cdot 10^6 \text{ m}^2)\) approximately five times the size of the study area. This would suggest direct estimates are strongly biased low and would account for the lower success in paternity assignment for Sirena, as the majority of fathers would be expected to reside outside the study area. Such a large estimate of neighborhood area suggests the effect of mating forays on gene flow may be even more pronounced. The resulting estimate of \(\hat{r} = 1,340\) m indicates mated individuals are separated by 30 roosting home range equivalents on average. Similarly, the genetic neighborhood area estimate suggests dispersal of male gametes across an area equaling 2,815 home range equivalents. This estimate is more than twice the neighborhood size predicted for Km23, and could have implications regarding habitat constraints on gene flow. The Sirena site is located within Corcovado National Park and is characterized by continuous stands of forest with abundant roosting habitat (i.e. \(Heliconia\) plants) in the understory. The Km23 site represents a forest remnant surrounded by a matrix of agricultural land. Habitat distribution, and resulting group roosting home range distributions, might influence the spatial scale over which breeding adults encounter one another during mating forays, ultimately determining the extent of gene flow. A better understanding of the relationship
between habitat suitability, social group distribution, foraging behavior, and mate choice could prove critical for the future conservation of this, and other highly philopatric species.

Inference regarding potential mating systems

A number of mating systems have been described within the order Chiroptera, including monogamy, promiscuity, and polygyny, with limited accounts of polyandry (McCracken and Wilkinson 2000). The observed patterns of mate choice and social structure of *T. tricolor* appear inconsistent with both monogamy, and the various forms of defense polygyny thought to occur in many neotropical species of tent-making bats (Kunz and McCracken 1996; McCracken and Wilkinson 2000). Lekking has been documented in paleotropical bats (Bradbury 1977; Wickler and Seibt 1976), with displays consisting of various forms of calling behavior. Vocalizations are important for maintaining group cohesion in *T. tricolor* (Chaverri et al. 2010), yet it is unclear if vocalizations serve a function in mating.

Two species of bat have promiscuous mating systems involving movement outside of roosting home ranges or extra-group copulations that resemble *T. tricolor*. Both sexes of brown long-eared bats, *Plecotus auritus*, show natal philopatry to summer maternity colonies (Entwistle et al. 2000), with offspring typically sired by males from other colonies (Burland et al. 2001). However, the social context in which mate choice occurs may be quite dissimilar to *T. tricolor*. The timing of oestrus in *P. auritus* is typical of most temperate bats species in that it coincides with seasonal migration to hibernation sites, and it is unclear whether mating occurs while bats are residing in maternity colonies, transient roosts, or hibernacula (i.e. *en masse* congregation of bats).
The African banana leaf-roosting bat *Neoromicia nanus* uses roosts similar to *T. tricolor*, but has a dissimilar social structure with groups consisting of multiple females with young, while males roost solitarily establishing small home ranges (Happold and Happold 1996). Happold and Happold (1996) described a prolonged mating season during which males were visited briefly by receptive females, and mating success was speculated to result from sperm competition. Sparse accounts of polyandrous mating in bats suggest that multiple matings by females may be important for some species (McCracken and Wilkinson 2000; Vonhof et al. 2006). In social structures where group mates are relatives, females may benefit from polyandry if they cannot avoid copulations with related males or distinguish kin from nonkin (Stockley et al. 1993). The patterns of mate choice we observed could be the result of sperm competition, or *in utero* or neonate mortality in response to close inbreeding (Keller and Waller 2002). Alternatively, the distribution of mated pairs may reflect kin recognition, where aggressive interactions between group members preclude close inbreeding and instigate forays in search of more suitable mates.

Social structure and mate choice patterns in *T. tricolor* most closely resemble those of pilot whales (*Globicephala melas*; Amos et al. 1993). Both sexes of *G. melas* are philopatric, pods represent matrilines, and breeding is thought to occur during short periods when males leave their natal pod in search of mates (i.e mating forays). The similarities in kin-group formation and mate selection in such divergent lineages raises interesting questions regarding potential evolutionary convergence on certain reproductive and dispersal strategies in response to similar ecological constraints, despite drastic differences in life history.
Conclusion

Few studies have examined the implications of extreme philopatry for mate choice and gene flow in non-cooperatively breeding mammals (Amos et al. 1993; Burland et al. 2001; Waser et al. 2012; Winters and Waser 2003). Here we present the most compelling example to date of the importance of mating forays as a behavioral adaptation to reduce close inbreeding and facilitate gene flow. Spix’s disk-winged bat displays a level of natal philopatry and site fidelity unique among even highly philopatric mammalian species. This study demonstrates that *T. tricolor* avoids close inbreeding by mating with non-group members separated by relatively large distances. This pattern of mate choice results in the dispersal of male gametes, moving genes well beyond group roosting home ranges, and maintaining gene flow in the absence of natal dispersal. All-offspring philopatry, small home range size, and mating forays create a pattern of spatial genetic structure in *T. tricolor* that is quite unique among mammals, more closely resembling plants with limited seed dispersal where gene flow is primarily mediated through dispersal of pollen. Mating forays also have significant implications for the social structure of this species. Because the underlying causes of natal dispersal (i.e. risk of inbreeding or competition for mates) are avoided due to extra-group breeding, both *T. tricolor* males and females are able to benefit from long-term associations within the natal group.

The results of this study indicate that male gamete dispersal is an important form of gene flow for this, and potentially other highly philopatric, gregarious species. Broadening our understanding of mating behaviors which reduce close inbreeding will improve our understanding of the variety of adaptive strategies that naturally occur across
species that are philopatric, naturally fragmented, or dispersal-limited. The apparent need for social groups distributed over relatively large geographic distances to facilitate outbreeding in this species could have conservation implications with regard to habitat loss and fragmentation. Future work should examine the relationship between mate choice and habitat distribution to predict the potential genetic consequences of habitat loss for this species.
References


Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices.


CHAPTER III

DEMOGRAPHIC AND GENETIC ESTIMATORS OF EFFECTIVE POPULATION SIZE \( (N_e) \) SUGGEST HIGH MALE REPRODUCTIVE SKEW IN SPIX’S DISK-WINGED BAT

Introduction

The concept of effective population size \( (N_e) \) was introduced by Wright (1931), and is defined as the size of an idealized population that experiences the same rate of change in allele frequencies as the biological population under consideration. In general, \( N_e \) is expected to be less than the actual population size \( (N) \). Different definitions of \( N_e \) exist, the two most common of which correspond to either changes in allele frequencies due to sampling variance associated with genetic drift (variance \( N_e \)), or decreases in heterozygosity following mating between relatives (inbreeding \( N_e \); Kimura and Crow 1963). Estimation of \( N_e \) is therefore important for understanding maintenance of genetic variation and adaptive potential, inbreeding depression, and the long-term viability of populations, and is of central importance in population and conservation genetics (Frankham 2005).

Most studies estimate the ratio of \( N_e/N \) rather than \( N_e \) for the purposes of inter-population or inter-species comparison. Theoretical considerations suggest that the \( N_e/N \) ratio across a single generation should fall within the range of 0.25–0.75 under most demographic conditions (Nunney 1996; Nunney and Elam 1994), falling below 0.25 only under extreme circumstances. However, empirical investigations of a wide range of plant
and animals species found that the ratio was 0.10–0.11 on average (Frankham 1995b; Palstra and Ruzzante 2008). Low $N_e/N$ has been attributed to fluctuating population size (Vucetich and Waite 1999; Vucetich et al. 1997), but more recently variation in reproductive success has been shown to be a major factor contributing to small $N_e/N$ (Hedrick 2005; Turner et al. 2002). In situations where a majority of reproduction is achieved by a small fraction of the population, $N_e$ can be much smaller than $N$.

Polygynous mating systems are a common feature of mammalian social structure (Clutton-Brock 1989). Under polygyny the number of breeding males is reduced, skewing representation of male ancestors in subsequent generations and theoretically lowering $N_e$ (Anthony and Blumstein 2000), yet the role polygyny plays in determining $N_e/N$ in wild populations has received little attention (Broquet et al. 2009; Kaeuffer et al. 2007). The majority of mating systems described for bats follow some form of polygyny, but such accounts are mainly based on behavioral studies without genetic data (McCracken and Wilkinson 2000). Few studies have examined the effects of mating system on $N_e$ for natural (Storz et al. 2001) or captive (Carroll and Mace 1988) populations of bats.

$N_e$ can be estimated demographically using life history data or with genetic data. Demographic methods of estimating $N_e$ are indirect, and infer the rate of genetic drift from parameters influencing variance in reproductive success. Demographic methods need information on sex ratio, age of sexual maturity, variance in breeding success of each sex, adult longevity, and variation in population size over time (Nunney 1993; Waples et al. 2011). Due to the logistical difficulty of obtaining such data, genetic methods have assumed an important role in estimating $N_e$ (Frankham 1995a). Genetic
methods provide the most direct and robust estimates for $N_e$ because they assess levels of
drift between generations, integrated over all possible causes. Several genetic methods
are available for $N_e$ estimation (Luikart et al. 2010). The most widely used and best
evaluated technique for measuring contemporary (i.e. recent, including the past one-to-
few generations) $N_e$ is the linkage disequilibrium (LD) method. The principle of the LD
method is that as $N_e$ decreases, genetic drift with few parents reduces the number of novel
allele combinations among different loci, i.e. linkage disequilibrium (Hill 1981).
Measuring the associations between alleles across several loci allows for the estimation
of inbreeding $N_e$. The LD method is easily implemented requiring a single sample of the
population, and recent simulation studies have demonstrated that this method provides
robust estimates for populations with overlapping generations (Robinson and Moyer 2013).

There have been few attempts to compare genetic and demographic methods for
$N_e$ estimation within the same population (e.g., Ardren and Kapuscinski 2003; Rowe and
Beebee 2004; Schmeller and Merila 2007). Among bats, there are relatively few
demographic (Carroll and Mace 1988; Storz et al. 2001) and no genetic estimates of
contemporary $N_e$. Our first objective was to generate genetic estimates of $N_e$ for two
populations of Spix’s disk-winged bat (*Thyroptera tricolor*) in order to calculate $N_e/N$
ratios. Using microsatellite genotypes we estimated $N_e$ using a single-sample LD method.
We then used these genetic estimates as a baseline with which demographic methods
could be compared. Both the mating system and potential skew in male reproductive
success are currently unknown for *T. tricolor*. Assessing individual reproductive success
in wild populations of bats proves challenging, due to their mobility and cryptic
behaviors. Our second objective was to test whether a polygynous mating system could be inferred via comparison of genetic and demographic estimates of $N_e$. We used demographic data obtained from capture-recapture analyses (Chaverri and Vonhof 2011; this dissertation) to parameterize a demographic estimator of $N_e$. We simulated $N_e$ under different levels of male reproductive variance until concordance with genetic estimates was reached, providing an indirect measure of male reproductive skew within each population and inference regarding the mating system.

Methods

*Ecology and life history of Thyroptera tricolor*

*Thyroptera tricolor* displays all-offspring philopatry, forming social groups consisting of one or more reproductive females and offspring of both sexes from multiple years (Chaverri and Kunz 2011). Population demography of *T. tricolor* has been previously investigated including sex ratios, ages of sexual maturation, annual survivorship and variance in female reproductive success (Chaverri and Vonhof 2011; this dissertation). Parentage analysis performed in conjunction with capture-recapture surveys indicate that mated individuals belong to different social groups whose roosting home ranges are separated by relatively large distances. Though males have been observed to mate multiply within years suggesting potential polygyny, the exact mating system of *T. tricolor* is unknown. Females produce a single pup per year and mortality is high, with $\sim 30\%$ of young dying before age six months. Annual survival increases significantly among older juveniles and adults, with mean lifespan estimated at approximately 3 years.
**Demographic and genetic data collection**

This study was conducted at two survey sites in southwestern Costa Rica during 2008 to 2011 (Fig. 4). We sampled 379 bats from 48 social groups at Km23, and 389 bats from 67 groups at Sirena. Survey areas for the two sites were approximately equal (93.6 ha for Km23 and 104.0 ha for Sirena). Capture locations were recorded on a hand-held GPS unit, and group roosting home ranges were constructed in ArcGIS 10.0 (ESRI Redlands, CA) using the 100% minimum convex polygon for the capture locations of all group members. Capture-recapture surveys indicated no movement of bats between populations, suggesting little potential for genetic estimates of $N_e$ to be biased due to immigration. DNA was extracted from samples of wing membrane tissue using Qiagen® DNeasy Blood & Tissue Kits and each bat was scored at eight microsatellite loci as described elsewhere (Chapter II). Heterozygosity estimates, allelic richness, concordance with Hardy–Weinberg equilibrium, and tests for linkage disequilibrium were assessed using the programs GENEPOP (Raymond and Rousset 1995) and FSTAT (Goudet 2001). We considered both sites to represent discrete genetic neighborhoods (Wright 1943) because each was geographically separate and each population was at Hardy–Weinberg equilibrium when assessed across the eight loci (Table 9).

To estimate adult population size, $N$, we used an indirect method for estimating genetic neighborhood size (i.e. the total number of bats occupying the neighborhood). Following Rousset (1997) we regressed pairwise genetic distances $F_{ST}/(1 - F_{ST})$ on the logarithm of geographical distances between all groups within a population. The inverse of the slope, $b$, of the regression line is an estimate of genetic neighborhood size. We multiplied the estimates of neighborhood size by the proportion of bats captured at each
site per annum that were adults. This provided an estimate of the number of adults of breeding age occupying the genetic neighborhood at each site, \( N \) for our purposes.

Table 9. Genetic diversity comparisons.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Km23</th>
<th>Sirena</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H_e )</td>
<td>0.817</td>
<td>0.801</td>
</tr>
<tr>
<td>( H_o )</td>
<td>0.821</td>
<td>0.798</td>
</tr>
<tr>
<td>( P_{95} )</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Allelic richness</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>HW disequilibria</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Linkage disequilibria</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

\( H_e \) = expected heterozygosity; \( H_o \) = observed heterozygosity; \( P_{95} \) = percentage of loci polymorphic at the 95\% criterion; HW disequilibria = number of loci not complying with Hardy–Weinberg expectations; Linkage disequilibria = number of loci combinations with significant correlations between allele frequencies.

**Genetic estimates of effective population size**

Genetic estimates of \( N_e \) were generated using a single-sample LD estimator implemented in the software package LDNE (Waples and Do 2008). We limited our analysis to a sample of reproductively mature adults, as recent simulation studies have demonstrated this approach is robust for estimating contemporary \( N_e \) in populations with overlapping generations (Robinson and Moyer 2013). Our sample consisted of 148 adult bats, captured throughout the four year study, from each site. Use of highly polymorphic loci with multiple low frequency alleles per locus can bias estimates of \( N_e \) provided by the LD method (Waples 2006). Given the size of our samples (\( n > 100 \)), we estimated \( N_e \) by excluding alleles at frequencies lower than 0.01 (\( P_{\text{crit}} = 0.01 \)) as recommended by Waples and Do (2010). For comparison with other studies of effective population size, we calculated a \( N_e/N \) ratio based on the genetic estimate.
Demographic estimates of effective population size

We estimated the effects of sex ratio, annual survival, and variance in reproductive success on $N_e$ using the minimal model for organisms with age-structure and overlapping generations (Nunney and Elam 1994). We simplified this model to accommodate an even sex ratio and no difference in survival among sexes (see below), resulting in

$$N_e = \frac{N \times T \times 4r^2}{2(rA(1 + l_A) + rI_{bm} + rI_{bf})}$$

where $T$ is mean generation time; $r$ the sex ratio; $A$ the mean adult lifespan; $l_A$ the standardized variance (variance/mean²) in lifespan; $I_{bm}$ the standardized variance in male reproductive success per breeding period; and $I_{bf}$ the standardized variance in female reproductive success per breeding period. All parameters were estimated using demographic and behavioral data as follows.

Sex ratio ($r$)

Sex ratios [$M/(M + F)$] for the two sites were not significantly different from parity ($Km23 = 0.54 \ [\chi^2 = 2.22, \ P = 0.136]$, Sirena = 0.48 $[\chi^2 = 0.51, \ P = 0.477]$). We therefore used a value of 0.5 to simplify our modeling.

Generation time ($T$)

This was calculated as $(M - 1) + 1/(1 - \phi)$ (Nunney and Elam 1994), where $M$ is the mean age of sexual maturity for the two sexes and $\phi$ is the mean annual survival probability. Males attain sexual maturity at one year of age, while females begin
reproducing after two years (Chaverri and Vonhof 2011). We therefore used a mean estimate of \( M = 1.5 \) years. Estimates of mean annual survival probabilities across three different study populations (Chapter 1) were 0.77 for prime age adults and did not differ by sex. Our assumed generation time \( (T) \) was therefore 3.4 years.

*Mean adult lifespans* (\( A \))

In Chapter 1 we demonstrated that *T. tricolor* exhibit a three age class survivorship curve characteristic of many mammals. Mean adult lifespan based on the assumption of age-dependent survivorship is therefore estimated as \( A = \left[ \frac{1}{(1 - \phi)} \right] (k + 1) / 2 \cdot k \), where \( k \) is the age of senescence (i.e. age after which mortality increases significantly; Nunney and Elam 1994). The parameter \( k \) was set to 3 based on age-structured annual survival probabilities inferred from capture-recapture modeling results (this dissertation). This results in a mean adult lifespan estimate of 2.9 years for both sexes. Standardized variance in lifespan \( (I_A) \) was calculated as \( I_A = \frac{A - k}{A \cdot k} \), equaling 0.012.

*Female variance in breeding success*

From Nunney and Elam (1994), \( I_{bf} = \frac{1 - \alpha_f}{\alpha_f} \), where \( \alpha_f \) equals the proportion of females producing offspring that survive to independence. In three well-studied populations in southwestern Costa Rica (Chaverri and Vonhof 2011), an average 30% of pups died within the first 3 months of life (i.e. prior to weaning). Capture-recapture modeling of age-structured survival probability indicates that survival increases dramatically after this period. We therefore assumed \( \sim 70\% \) of females successfully produced offspring surviving to independence in any particular year, and estimated \( \alpha_f \) at 0.7. This results in an \( I_{bf} \) estimate of 0.43.
Assessing variance in male breeding success

For $I_{bm}$ we assumed a lottery polygyny breeding system in which females mate no more than once per season but all males attempt to mate many times. In this situation, $I_{bm} = R + (1 - \alpha_m)/\alpha_m$ (Nunney and Elam 1994), where $\alpha_m = \text{proportion of males attempting to breed}$, and $R = r / (r \cdot \alpha_f)$. We have no empirical data to aid in estimation of $\alpha_m$, and this parameter is the main focus of this investigation.

We initially held all other parameters constant (as described above) and considered this the “typical population” model for *T. tricolor*. Extra variance among males in reproductive success would be expected to reduce $N_e$. Thus $\alpha_m$ should reflect the overall level of polygyny within the population. We simulated different levels of male reproductive skew (i.e. trying a range of values [0.05–1.0] for $\alpha_m$, and therefore $I_{bm}$). We compared $N_e$ estimates from the genetic estimator to the demographic estimates to investigate the effects of varying male reproductive success on effective population size over the period 2008–2011. This iterative process was continued until concordance between the estimates was reached.

Assuming demographic estimates could be biased by inaccuracy of other parameters within the model, we tested the sensitivity of the model to different values of (1) proportion of successful female breeders $\alpha_f$, (2) annual survival probability $\phi$, and (3) the age of senescence $k$. Sensitivity analyses were limited to these factors, as they represent the only other independent parameters in the model. We varied the values of each individually in an attempt to achieve similar concordance with the genetic estimates. At the same time we set $\alpha_m$ to 0.25, 0.5, and 0.75 to explore model sensitivity to each of these parameters over a realistic and wide range of male reproductive success.
Temporal variance in effective population size

Accurate estimates of $N_e$ require consideration of potential temporal variation in $N$, and the resultant change in the genetic composition of populations due to drift in allele frequencies (Hansen et al. 2002; Hoffman et al. 2004; Vucetich and Waite 1999). Thus, the temporal stability of population size, both $N$ and $N_e$ is of concern over long time scales. Our aim was not to provide long-term estimates of $N_e$, but rather to quantify the relative importance of reproductive strategies on contemporary $N_e$ (i.e. 1 to 2 generations). This was accomplished by generating estimates based on a common temporal scale, representing 1 to 2 generations in length.

Single-sample LD genetic estimators estimate contemporary $N_e$ for one-to-few generations (Luikart et al. 2010), and our demographic estimates of mean generation time (3.4 years) indicate that approximately two generations would have been sampled during our study. In addition, the indirect estimates of genetic neighborhood size, $N$, were calculated from four years of samples and should represent temporal averages for approximately 1 to 2 generations.

Results

Genetic diversity of T. tricolor populations

Genetic diversity estimates at the eight microsatellite loci are summarized in Table 9 for the two populations. We assume there were negligible effects of marker locus mutation or selection on the allele frequency distributions that we measured. All eight loci were highly polymorphic. Genetic diversity at the microsatellite loci in our populations was high, with a maximum of 23 alleles per locus. Observed heterozygosity was slightly higher at Km23, while allelic richness was higher at Sirena. There were no
instances of failure to comply with Hardy–Weinberg expectations and only two instances of linkage disequilibria among the sample sets.

**Genetic estimates of \( N_e \)**

The \( N_e \) estimate from LDNE for Km23 was 102 (95% CI 88–119; Table 10) based on a sample of reproductive adults (\( n = 148 \)) during the period 2008–2011. The indirect estimate of genetic neighborhood size indicated that mean adult population size from 2008–2011 was 241, resulting in a \( N_e/N \) ratio of 0.42. The genetic \( N_e \) estimate for Sirena was 183 (149–233). The indirect estimate of genetic neighborhood size indicated a mean adult population size of 656, resulting in a \( N_e/N \) ratio of 0.28. The linkage-disequilibrium method yielded a higher estimate of \( N_e \) for the Sirena population, which corresponded well with the larger estimate of \( N \) resulting from the genetic neighborhood size calculation. This suggests that the populations of *T. tricolor* were more accurately approximated by the genetic neighborhood rather than the census size of each study site.

The rate of loss of genetic variability within a population by genetic drift can be quantified as \( 1/2N_e \) per generation. Thus, the predicted rate of drift was 0.0049 for Km23. The predicted rate of drift for Sirena was 0.0027, suggesting that loss of heterozygosity per generation is low at both sites.

Table 10. Estimates of genetic neighborhood sizes, genetic and demographic estimates of effective population sizes, and skew in male reproductive success.

<table>
<thead>
<tr>
<th>Method</th>
<th>Population</th>
<th>Km23 (95% CI)</th>
<th>Sirena (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic neighborhood size (( N ))</td>
<td></td>
<td>241</td>
<td>656</td>
</tr>
<tr>
<td>( N_e ) – genetic estimate</td>
<td></td>
<td>102 [88–119]</td>
<td>183 [149–233]</td>
</tr>
<tr>
<td>( N_e/N )</td>
<td></td>
<td>0.42</td>
<td>0.28</td>
</tr>
<tr>
<td>Proportion of successful male breeders (( \alpha_m ))</td>
<td></td>
<td>0.107</td>
<td>0.0565</td>
</tr>
<tr>
<td>Variance in male breeding success (( I_{bm} ))</td>
<td></td>
<td>9.77</td>
<td>18.13</td>
</tr>
</tbody>
</table>

Brackets show 95% confidence limits. Estimates of \( \alpha_m \) and \( I_{bm} \) represent values necessary to achieve concordance between the genetic and demographic estimates of \( N_e \).
Demographic estimates of $N_e$

We calculated demographic estimates of $N_e$ for *T. tricolor* based on varying levels of breeding success among males in what we considered a typical population (see methods). Demographic estimates of $N_e$ greatly exceeded the 95% upper confidence limit of the genetic estimates obtained using the linkage disequilibrium method unless the proportion of males successfully reproducing was low ($\alpha_m \approx 0.05–0.1$; Fig. 11). This was assumed to be because variance in male breeding success $I_{bm}$ was grossly underestimated. Values of $\alpha_m$ which achieved concordance between the estimates were 0.107 for Km23 and 0.057 for Sirena (Table 10). These values result in $I_{bm}$ estimates of 9.77 for Km23 and 18.13 for Sirena, suggesting 10% or less of males may achieve reproductive success annually within a typical population of *T. tricolor*.

![Graph](https://via.placeholder.com/150)

**Figure 11.** Relationship between effective population size $N_e$ and the proportion of males successfully reproducing, $\alpha_m$ based on demographic modeling for the (a) Km23 and (b) Sirena populations. Genetic estimates of $N_e$ (dashed lines) with 95% CIs (dotted lines) are provided for reference. Parameterization of the underlying demographic model follows that for the “typical” population of *T. tricolor* as described in the methods.
Sensitivity of the model to female reproductive success indicated that comparable demographic and genetic estimates could only be obtained with values of $a_f < 0.2$ (i.e. $\sim 20\%$ of females reproducing) for both populations (Fig. 12). Such low values of female reproduction seem unrealistic from the standpoint of population dynamics and viability, and are inconsistent with field observations (Chaverri and Vonhof 2011; this dissertation). Results of sensitivity analysis to annual survival probability $\phi$ differed between the two sites. The model for Km23 was more sensitive to $\phi$, and values of $N_e$ similar to genetic estimates were possible below survival values of approximately 0.5 (Fig. 13a) when the proportion of males reproducing was low ($a_m \approx 0.25$). The model for Sirena was relatively insensitive to survival rate (Fig. 13b). No values of age of senescence ($k$; Fig. 14) could create concordance between the demographic and genetic $N_e$ estimates under the range of male reproductive success examined (i.e. $a_m = 0.25$–0.75).

Figure 12. Demographic model $N_e$ sensitivity to the proportion of females successfully breeding $a_f$ for the (a) Km23 and (b) Sirena populations. Simulations were conducted over a range of values for male breeding success, $a_m = 0.25$, 0.5, and 0.75. Genetic estimates of $N_e$ (dashed lines) with 95% CIs (dotted lines) are provided for reference.
Figure 13. Demographic model $N_e$ sensitivity to the annual adult survival probability $\phi$ for the (a) Km23 and (b) Sirena populations. Simulations were conducted over a range of values for male breeding success, $\alpha_m = 0.25$, 0.5, and 0.75. Genetic estimates of $N_e$ (dashed lines) with 95% CIs (dotted lines) are provided for reference.

Figure 14. Demographic model $N_e$ sensitivity to average age of senescence $k$ for the (a) Km23 and (b) Sirena populations. Simulations were conducted over a range of values for male breeding success, $\alpha_m = 0.25$, 0.5, and 0.75. Genetic estimates of $N_e$ (dashed lines) with 95% CIs (dotted lines) are provided for reference.
The level of polygyny, as inferred from the standardized variance in male reproductive success based on reconciled demographic and genetic estimates $I_{bm}$, differed among the populations. We observed weaker polygyny ($I_{bm} = 9.77$) in the smaller Km23 population ($N = 241$), resulting in a larger $N_e/N$ ratio (0.42; Table 10). Conversely, polygyny was greater in the larger population. We observed higher polygyny ($I_{bm} = 18.13$) in the Sirena population ($N = 656$), resulting in a smaller $N_e/N$ ratio (0.28). Similar observations of stronger polygyny in larger populations have been made in frogs, and this pattern potentially explains the low $N_e/N$ ratios commonly observed for many amphibian species (Ficetola et al. 2010).

Large variances in male reproductive success when compared to females are the result of sexual selection, with this phenomenon commonly referred to as Bateman’s Principle (Bateman 1948). The difference between the sexes determines the intensity of sexual selection, which can be quantified as the ratio of male reproductive variance $I_{bm}$ to female reproductive variance $I_{bf}$, or $I_{bm}/I_{bf}$. The opportunity for sexual selection in the study populations, estimated as the ratio $I_{bm}/I_{bf}$ (using the standardized variance in female reproductive success from the typical population model, $I_{bf} = 0.43$), was $9.77/0.43 = 22.7$ for Km23 and $18.13/0.43 = 42.2$ for Sirena. Values greater than one signify that males experience a greater force of sexual selection.

Discussion

Within a polygynous mating system, males compete for access to reproductive females, or females show preference for some males over others. The resulting sex difference in reproductive success is known as Bateman’s Principle (Bateman 1948). This nonrandom sampling of the adult male gamete pool in each generation increases the rate
of genetic drift. We searched for evidence of this sampling process by comparing genetic estimates of $N_e$ to a demographic model based on empirical life history data. We found relatively low genetic estimates of $N_e$ relative to adult population size, $N$. Demographic estimates of *T. tricolor* effective population sizes were consistently higher than genetic estimates, and only fell within the 95% confidence limits of genetic estimates when variance in reproductive success was large for either sex, but particularly for males. The low rates of female breeding success necessary to reconcile the estimates initially seem unrealistic from the perspective of population dynamics and viability (but see below). Polygyny can account for discrepancies between genetic and demographic estimates at both populations, and seems to be a major influence over contemporary $N_e$ (< two bat generations). Therefore, our results suggest that the two natural populations of *T. tricolor* are characterized by high variance in male mating success.

*Comparison of $N_e/N$ ratios to theoretical expectations*

It has been hypothesized that high variance in male reproductive success typical of polygyny (i.e. harem formation or lekking) might represent extreme circumstances capable of reducing the $N_e/N$ ratio below theoretical expectations (Storz et al. 2001). Our demographic modeling suggests $N_e/N$ ratios for our two populations were substantially lower than would be expected if male-skew were absent. Yet, despite evidence for high variance in male mating success, the $N_e/N$ ratios were within the 0.25–0.75 range expected under unexceptional demographic conditions (Nunney and Elam 1994). This outcome is similar to Storz et al. (2001) who also used the minimal demographic model of Nunney and Elem (1994) to estimate $N_e/N$ in a single population of greater short-nosed fruit bats (*Cynopterus sphinx*), a species with a well-documented mating system of harem
polygyny. Their estimate of 0.47 is slightly higher than our estimates from two populations of *T. tricolor* (0.28 and 0.42). Our results are also similar to the only other study of bats available in the literature, also having polygynous mating system. Carroll and Mace (1988) estimated $N_e/N$ ratios of 0.18–0.43 for a captive population of Rodrigues fruit bat (*Pteropus rodricensis*). Estimates from these three species suggest that $N_e/N$ ratios are high (0.36 on average) among bats, even in species with confirmed polygyny. These observations generally support the notion that the influence of polygyny on genetic drift in bats may be mitigated by overlap of generations and continual turnover of breeding males. Storz et al. (2001) attributed the relatively high $N_e/N$ to the disproportionately short sexual maturation period in bats when compared to adult lifespan, concluding that the influence of polygynous mating on $N_e/N$ was mitigated by extensive overlap of generations. Our results may be applicable to a number of species characterized by polygynous mating systems and overlapping generations.

*Potential for genetic compensation*

Several empirical studies of various polygynous species have observed the situation where a greater percentage of adults successfully reproduce when population size is small (reviewed in Palstra and Ruzzante 2008). This phenomenon, referred to as genetic compensation, results in a larger $N_e/N$ ratio when population size is small and a lower $N_e/N$ ratio when population size is large due to an assumed positive correlation between the variance in male reproductive success and population size (Ardren and Kapuscinski 2003). Thus, in large populations, fewer successful males appear to monopolize access to females. Genetic compensation via polygyny may be responsible for the differences observed between our populations of *T. tricolor*, and our results
suggest the possibility of a joint effect of population size and polygyny on $N_e$.

Interestingly, demographic simulations of sage grouse (Stiver et al. 2008) suggest that $N_e/N$ ratios decrease as $N$ increases in lek mating systems. This occurs because lek size increases with $N$, inflating the variance as a smaller proportion of males increase their mating success. The mating system of $T. \text{tricolor}$ is unknown, but the social system of this species is inconsistent with harem or resource defense polygyny. Lekking may represent a compatible polygynous mating system for this species. The importance of population size, mating system, and the possibility of genetic compensation to maintain genetic diversity in this, and other more threatened bat species merits future investigation.

*Intensity of sexual selection and inference regarding $N_e$*

Male reproductive variance was large at both sites ($I_{bm} = 9.77$ at Km23 and 18.13 at Sirena). Such high levels of variance are surprising given the lack of apparent sexual dimorphism in this species. The resulting $I_{bm}/I_{bf}$ ratios were high, suggesting a high level of sexual selection among males and a low level of sexual selection on females ($I_{bf} = 0.43$). This is logical as most females are successful, having one offspring per year. Our calculated $I_{bm}/I_{bf}$ values are among the highest observed for promiscuous mammals (mean $= 6.82$; Garg et al. 2012) and suggest potential overestimation of $I_{bm}$ (see below).

*Underestimation of variance in female breeding success and implications for $N_e$*

It is important to note that the observed discrepancies between genetic and demographic estimates may be due to high variance in reproductive success among both sexes. Demographic estimators often overestimate $N_e$ because they fail to account for variance in reproductive success beyond Poisson expectations, which reduces $N_e$ compared to $N$ (Rowe and Beebee 2004; Schmeller and Merila 2007). Our estimate of
variance in female breeding success ($I_{bf}$) for *T. tricolor* of 0.42 was within the range of similar estimates for birds and mammals (0.14–0.64) based on fledglings or weaned young (Nunney 1996). However, Rowe and Beebee (2004) point out that error in estimating variance in female breeding success represents a likely source of bias when calculating the $N_e/N$ ratio demographically. In the minimal demographic model, $I_{bf}$ reflects variance in breeding success only to the point of independence from the mother. In many species, however, variance may be high well beyond this point in life history, and it is of course the full variance up to attainment of sexual maturity in progeny that affects $N_e$. Such information is generally hard to obtain, and was a major reason for the more tractable measure (breeding variation to the point of independence from the mother) advocated by Nunney and Elam (1994). The discrepancies we observed between demographic and genetic $N_e$ estimates may highlight the sensitivity of the minimal demographic model to this underlying assumption. Though our mean lifespan estimates suggest each female may take part in up to two reproductive seasons during its lifetime, offering the possibility to make up for unsuccessful years, recent work has indicated that variance in female reproductive success caused by female breeding failure can have significant impacts on $N_e$ (Stiver et al. 2008). Considering our $I_{bm}$ values represent indirect estimates of male reproductive success based on model simulations, and that the observed $I_{bm}/I_{bf}$ values are among the largest recorded, it seems likely that $N_e$ is the result of greater variance in reproductive success among both sexes. We conclude that though high variance in male breeding success is apparent in *T. tricolor*, the degree of polygyny we observed may be overestimated due to an underestimation of variance in female breeding success.
Sensitivity of demographic estimates to annual survival

The discrepancy between genetic and demographic estimates of $N_e$ in *T. tricolor* was highest when variance in male reproductive success was low. However, at Km23 the estimates could be reconciled by a 35% reduction in annual survival with moderate variance in male breeding success ($\alpha_m = 0.25$; Fig. 13a). Such a low level of survival falls outside of the 95% confidence interval for this estimate (0.62–0.87) based on capture-recapture modeling of 5 years of data (this dissertation). Further, the demographic model for the Sirena population was largely insensitive to the effect of survival on $N_e$ (Fig. 13b), suggesting it is unlikely we are systematically overestimating annual survival. Though we acknowledge the importance of annual survival on $N_e$, we do not accept that low $N_e/N$ ratios could result from overestimation of survival without a large degree of variance in male breeding success.

Study design and potential sources of bias

By conducting our study at the scale of the genetic neighborhood we addresses three major sources of bias in estimating $N_e$, namely temporal variation in population size, gene flow, and population substructure. The single largest determinant of $N_e$ is temporal fluctuation in population size (Frankham 1995b; Vucetich and Waite 1999; Wright 1938). Our indirect estimates of genetic neighborhood size, $N$, represent temporal averages for 2008–2011 at each site. These allowed for demographic estimates of contemporary $N_e$ which accounted for temporal fluctuations in population size. The genetic neighborhood is fundamental to the isolation-by-distance model of gene flow, and approximates the spatial extent of panmixia when organisms are continuously distributed (Wright 1943). Therefore genetic neighborhood and the number of adults occupying it,
rather than survey area and census size, represents a more appropriate scale for evaluating the effects of drift, gene flow, and genetic spatial structure on these populations. Gene flow within *T. tricolor* has been shown occur over local spatial scales, with the dispersal of male gametes occurring during extra-group matings (this dissertation). In addition, populations of *T. tricolor* appear to consist of kin-based groups differentiated by an isolation-by-distance pattern of genetic spatial structure (this dissertation). It is therefore unlikely that immigration from outside populations or cryptic genetic structure within populations would bias \( N_e \) low. Other factors which could influence variance in reproductive success among *T. tricolor* warrant further investigation, including the effect of social group size, and the importance of habitat patch quality considering the high level of philopatry among both sexes.

*Possibility of a promiscuous mating system*

Our reconciliation of genetic and demographic \( N_e \) estimates in *T. tricolor* is inferential because we have no direct measurements of male reproductive success, or the success of offspring to reach sexual maturity in the next generation. Similarly our deduction that the mating system of *T. tricolor* represents polygyny is also inferential. It is important to note that variance in male reproductive success generated by a promiscuous mating system can be similar to a polygynous mating system if cryptic female choice or sperm competition is present, thereby increasing variance in male reproductive success (Preston et al. 2003). Despite a large literature suggesting predominantly polygynous mating systems among bats (reviewed in McCracken and Wilkinson 2000), growing evidence suggests that promiscuity and opportunities for cryptic mate choice may be prevalent (Chaverri et al. 2008; Garg et al. 2012; Vonhof et
Thus our results could be consistent with a promiscuous (i.e. polygynandrous) mating system as well. We conclude that *T. tricolor* appear to have a mating system characterized by variance in reproductive success among males, where a reduced number of males have the opportunity to mate.

**Conclusion**

Our study represents one of only three attempts to estimate contemporary $N_e$ for a bat species, and surprisingly is the first to do so using genetic methods. As such, this is also the first study to compare demographic and genetic estimators of $N_e$ within a population of bats. In addition, this comparison was made for two study populations, allowing for inter-population comparisons of $N_e/N$, proportion of males successfully reproducing $\alpha_m$, and variance in male reproductive success $I_{bm}$. Such multi-population comparisons increase our understanding of the full range of values these important parameters can assume under natural conditions, and the potential for spatial variance in demographic rates that influence $N_e$. Future application of these techniques should include multiple populations to account for such variation. The difference in our estimates illustrates the critical importance of sampling multiple populations to extract maximum information.

Population genetics are increasingly well studied in bat species, but attempts to integrate such studies with behavioral and demographic data into a cohesive picture for the purpose of conservation are nonexistent. This may be due to the difficulties associated with assessing behavior in often cryptic bat species and the logistical difficulties associated with longitudinal demographic studies. A key topic for bat conservation is understanding how mating systems may vary in bat populations of different size and the
way this can affect genetic diversity. While accurate measures of $N_e$ are highly relevant for conservation, their potential for explaining important life history characteristics and evolutionary outcomes should not be overlooked. The methodology outlined here provides important insight into the evolutionary dynamics of $T. tricolor$ populations, and may prove useful for other cryptic species where it is difficult to observe multiple matings and litters consist of one offspring. It is increasingly important to improve our understanding of $N_e$ estimators and their application, not only in the conservation of small populations where the prospect of extinction from stochastic processes is often high, but also for increased understanding of the effects of reproductive success on population processes.
References


Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indicies.


Appendix

Western Michigan University Institutional Animal Care and Use Committee
Date: May 19, 2008

To: Maarten Vonhof, Principal Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol No. 08-05-02

Your protocol entitled “Social Behavior and Metapopulation Dynamics of Thyroptera Tricolor” has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: May 14, 2009
Date: May 11, 2011

To: Maarten Vonhof, Principal Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol No. 11-05-04

Your protocol entitled “Social Behavior and Population Genetics, and Demography of Thyroptera Tricolor” has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: May 11, 2012