

2014

Relatedness of Male Hoary Bats at a Migratory Stopover Site in Northwestern California

April K. Kaneshiro

Grand Valley State University, kaneshia@mail.gvsu.edu

Follow this and additional works at: <http://scholarworks.gvsu.edu/sss>

 Part of the [Animal Sciences Commons](#)

Recommended Citation

Kaneshiro, April K., "Relatedness of Male Hoary Bats at a Migratory Stopover Site in Northwestern California" (2014). *Student Summer Scholars*. 135.

<http://scholarworks.gvsu.edu/sss/135>

This Open Access is brought to you for free and open access by the Undergraduate Research and Creative Practice at ScholarWorks@GVSU. It has been accepted for inclusion in Student Summer Scholars by an authorized administrator of ScholarWorks@GVSU. For more information, please contact scholarworks@gvsu.edu.

Relatedness of male hoary bats at a migratory stopover site in northwestern California

April K. Kaneshiro, student researcher

Amy L. Russell, faculty mentor

Abstract

The social structure and genetic make-up of the hoary bat (*Lasiurus cinereus*) during its fall migration is not well known. Recently, pairs of male hoary bats were observed flying together in the Humboldt Redwoods during the autumn migration. When one individual was netted, the other member of the pair circled around and remained in the area while the two bats called to one another. This suggests a strong social, and a possible genetic, bond between the two males. In order to determine whether these males were related, wing tissue samples were collected from 15 pairs and 76 singleton individuals from the general migrating population. All but two individuals were genotyped at 14 microsatellite loci. The genetic relatedness analysis revealed that although the behaviorally interacting pairs were not more related than the general population, there was a wide range of relatedness within the population ranging from 0.0 to 1.0. Unexpectedly, 31 singleton individuals were found to have high relatedness values with another individual in the population that indicated a full-sibling or parent-offspring relationship. Furthermore, five pairs of singleton individuals possessed relatedness values of 1.0 that reveal an identical twin relationship. Altogether, our results suggest that this area is part of a commonly used migration route for male hoary bats and that this migratory behavior is shared within families even if the individuals are not observed exhibiting co-migratory behavior.

Introduction

A behavior generally evolves in a species because of the benefits incurred by specific actions. However, some organisms possess apparently self-sacrificing behaviors that may seem unlikely to have persisted in the face of evolution due to the negative effect on the individual's direct fitness (Bourke 2014). Direct fitness is defined as the number of offspring produced by an individual, and is contrasted with indirect fitness, which is the number of offspring produced by close relatives of the individual. Altruistic behaviors are explained as having evolved due to the improvement in the organism's indirect fitness as described by Hamilton's (1964) rule. For example, cooperative courtship has been observed in the males of the wild turkey (*Meleagris gallopavo*). Krakauer (2005) followed six coalitions composed of five male pairs and one four-member group for five years. Within each coalition, there was a dominant male that mated with the females and a subordinate or helper male that helped to attract and guard the females. The results of this study revealed that members of the same coalition had significantly higher relatedness values, generally on the order of half- or full-siblings, than the general population. As a result, both the dominant and subordinate males benefitted from the coalition, with dominant males increasing their direct fitness by fathering more offspring and subordinate males increasing their indirect fitness by assisting a close relative in producing more offspring (Krakauer 2005). This is not unique; cases of altruism have been reported in various species of insects and birds (Bourke 2014).

One behavior that appears in many species is migration. Although migration can have various definitions based on the organism being studied (Dingle and Drake 2007), migration will be defined here as the seasonal movement of a group of organisms to a location that possesses better conditions such as available shelter or more abundant food sources. For many organisms, migration is a behavior necessary for survival. However, there are many costs associated with migration. It can require a great amount of energy, increase the risk of predation, and does not guarantee survival (Moussy et al. 2013). For juveniles, migration can be especially costly due to their lack of experience and shorter time to prepare for the journey.

Relatedness among migrating individuals has been shown to influence migratory behavior. Colbeck et al. (2013) found that beluga whales (*Delphinapterus leucas*) located in and around the Hudson Bay migrate to summering areas with close kin such as their parents or siblings, as well as with less closely related relatives. However, this is not true for all animals. For bats, there appears to be little correlation between migration and relatedness (Burland et al. 2001; Kerth et al. 2002). Nevertheless, bats do exhibit some general trends in migration. Migration is observed more in temperate, tree-roosting bats than in temperate, cave-dwelling bats due to the exposure of the former to more extremes of temperature (Moussy et al. 2013). In addition, temperate bats tend to migrate in order to prepare for hibernation during the winter (Fleming and Eby 2003), whereas tropical bats appear to migrate in response to fluctuations in food availability (Moussy et al. 2013). Lastly, migration behaviors are often sex-biased, with females being more likely to migrate and migrating greater distances than males (Fleming and Eby 2003; Moussy et al. 2013), which is likely due to their need to find suitable roosts and resources for their offspring.

The hoary bat (*Lasiurus cinereus*) is a tree-roosting bat that is found throughout the Americas. In North America, it undergoes long-distance migration to and from wintering grounds in the fall and spring respectively. During the summer, the hoary bat resides in North America with males usually inhabiting the mountainous western areas and females residing farther east (Cryan 2003). Copulation is believed to occur mainly during the autumn migration (Shump and Shump 1982), and females usually migrate prior to parturition and offspring-rearing during the spring (Krauel and McCracken 2013).

During the autumn migration, pairs of hoary bats in California were observed flying together in an area of the redwood forest called the Humboldt Redwoods (Weller and Giordano 2013). When both members of the pair were caught, they were found to be males. Furthermore, when only one of the bats involved in a pair was netted, the free individual remained in the area and the two bats called to each other. This suggests that these males share a social bond. Because hoary bats usually produce two pups per litter (Shump and Shump 1982), we hypothesize that these co-migrating males are related. There are possible benefits to migrating with a relative such as increasing indirect fitness by assisting kin in safe migration or forming male reproductive coalitions for mating during migration (Díaz-Muñoz et al. 2014). Nevertheless, there is also the possibility that these males are not related and are behaving altruistically. Although rare, altruistic behavior between non-kin has been reported in the common vampire bat *Desmodus rotundus* (Carter and Wilkinson 2013). In order to gain insight into this new behavior, a genetic analysis of relatedness was performed to assess whether the co-migrating males were significantly more related than random pairs of individuals in the general migrating population.

Methods

Study site and field methods

Field work for this study was conducted from early June 2013 to mid-January 2014 along a 5 km stretch of the Bull Creek located in the Humboldt Redwoods State Park, Humboldt County, California. The study site was divided into four sub-units referred to as Bull Creek Albee, Bull Creek - Bridge, Blue Slide, and Bull Creek - Shoo Fly (Table1). *Lasiurus cinereus* were caught during the night using standard 2.6 mm mist nets in a triple-high configuration with three standard mist nets stacked on top of one another (Kunz et al. 2009). All bats were sexed and aged upon capture, and tissue samples consisting of 3 mm wing biopsies were collected from the left wing of all netted bats (Worthington Wilmer and Barratt 1996). A total of 112 individuals including 11 known pairs and 4 possible pairs were used for this study. Known pairs were defined as two individuals that were netted at the same time, while possible pairs were defined as groups of bats (>2 individuals) that were netted at the same time.

Table 1. Information about the site sub-units and number of individuals and pairs captured at each location.

Site Sub-Unit Name	Dates of Site Sub-Unit Usage	Number of Individuals Captured	Number of Pairs Captured
Bull Creek Albee	June 11 October 4 November 13	44	7
Bull Creek - Bridge	September 19 October 5 October 22	43	6
Blue Slide	October 6 November 22 January 15	15	2
Bull Creek - Shoo Fly	October 14 November 6	10	None

DNA extraction and genotyping

Tissue samples were preserved with either silica bead desiccant or a 20% DMSO salt saturated solution. Total genomic DNA was extracted using a DNeasy® Blood and Tissue kit (Qiagen) and then PCR amplified for 15 microsatellite markers: Coto_G12F_B11R (Piaggio et al. 2009), IBat CA22 (Oyler-McCance and Fike 2011), MS1C01 and MS3E10 (Trujillo and Amelon 2009), and LboD266, LboD200, LboD230, LboC07, LboD203, LboD204, LboD248, LboB06, LboD240, LboD245, and LboD08 (Eackles and King, pers. comm.). PCR amplifications were qualitatively confirmed using gel electrophoresis prior to being sent to the University of Arizona for fragment analysis. Microsatellite alleles were called and binned using the Geneious software (Biomatters Limited). Genepop (Rousset 2008) was used to detect deviations from Hardy Weinberg equilibrium. Lastly, the presence of null alleles was analyzed by MicroChecker (Van Oosterhout et al. 2004).

Relatedness analysis

The program ML-Relate (Kalinowski et al. 2006) was used to calculate all pairwise relatedness (R) values for the sample population. In order to determine if the behaviorally interacting pairs were significantly related, we performed likelihood ratio tests in ML-Relate with 10,000 simulations, in which an unrelated relationship (the putative hypothesis) was compared to a half-sibling relationship (the alternative hypothesis) for each pair. The p value given for each likelihood ratio test is the probability that the alternative hypothesis was a valid explanation for each pairs' R value. If the p value was less than 0.05, the alternative hypothesis could be rejected in favor of the putative hypothesis.

Results

All but two of the 112 individuals were successfully genotyped at 14 of the 15 microsatellite loci. The IBat CA22 locus was excluded due to ambiguous peaks when analyzed in Geneious. Four additional loci (LboC07, MS3E10, MS1C01, and LboD240) were excluded for having null alleles as detected by deviations from Hardy Weinberg equilibrium found by Genepop (Table 2; Rousset 2008) and confirmed by MicroChecker (Van Oosterhout et al. 2004).

Table 2. Number of expected and observed heterozygosity and F_{IS} values for each microsatellite locus.

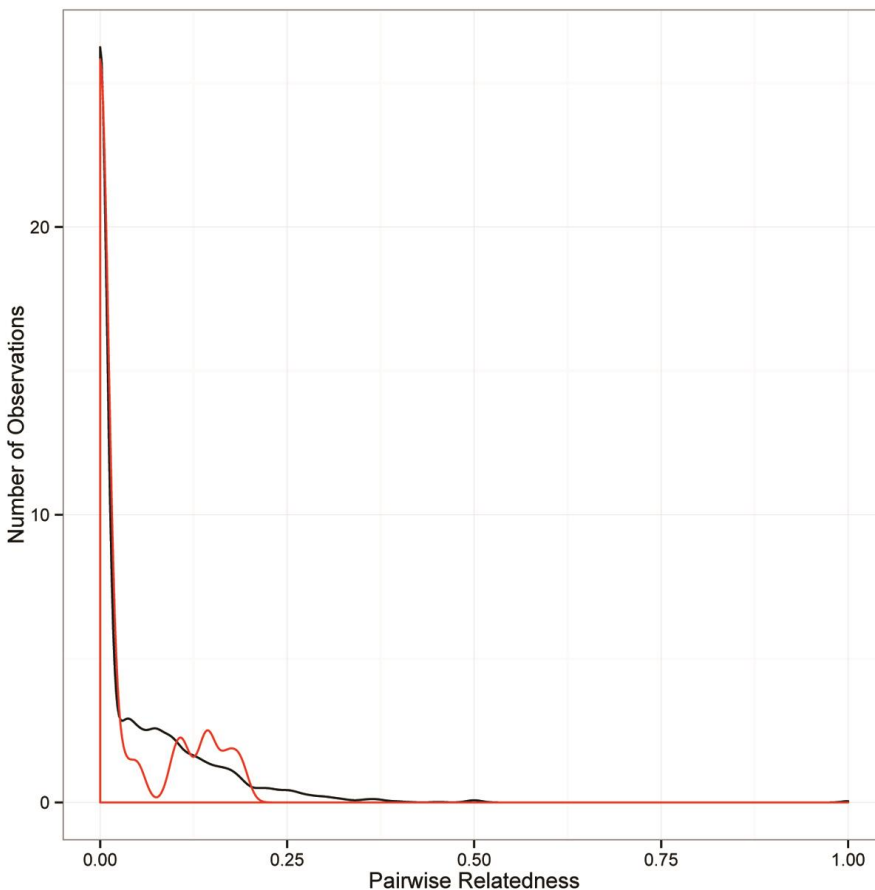
Locus	Number of Alleles	Number of Individuals Successfully Genotyped	Expected Heterozygosity	Observed Heterozygosity	F_{IS} Value
Coto_G12F_B11R	20	103	0.886	0.864	0.0253
LboD200	13	103	0.837	0.777	0.0726
LboC07	6	100	0.724	0.410	0.4346
LboD266	65	103	0.981	0.971	0.0106
LboD230	11	103	0.761	0.816	-0.0716
LboD203	9	103	0.740	0.767	-0.0364
LboD248	10	103	0.824	0.796	0.0342
LboD204	8	103	0.730	0.796	-0.0918
LboB06	4	103	0.111	0.097	0.1278
MS3E10	14	103	0.886	0.631	0.2889
LboD08	11	102	0.836	0.863	-0.0326
LboD245	7	103	0.731	0.738	-0.0095
MS1C01	13	103	0.803	0.660	0.1786
LboD240	14	103	0.867	0.786	0.0934

The sample population had a sex ratio of 108 males to four females. All sampled individuals were adults except one young of the year. All known pairs were composed of two adult males except for one pair comprised of an adult male and an adult female. All possible pairs were comprised of adult males except for one group that contained a juvenile male. Three possible pairs contained three individuals and the fourth consisted of five individuals. In addition, 10 netted individuals in the sample population were suspected to be recaptured individuals due to

evidence of recent wing biopsies found on their left wings. Seven of the suspected 10 were genetically identical ($R = 1.0$) to another individual that had been netted earlier. This confirmed that these suspected individuals had already been included in the sample, so samples representing recaptures of these seven individuals were omitted from the dataset. The highest relatedness values for the other three suspected recaptures were less than 0.32, which indicated that they had not been included in the study previously. Therefore, these three were kept in the dataset.

The average pairwise relatedness was $R = 0.047$ for the total migrating population, $R = 0.043$ for the known pairs, and $R = 0.039$ for the possible pairs. A Mann-Whitney U test was performed to determine if the distribution of relatedness values of the known pairs and the possible pairs were significantly different from the distribution of relatedness values of the general population (Figure 1). The calculated W value was 82455.5 and the p value was 0.2001. This confirmed that the relatedness distributions were not significantly different.

Figure 1. Distribution plot of the relatedness values of the known and possible pairs compared to the relatedness values of the general population. Total migrating population in black; behaviorally interacting pairs in red.



The relatedness values of the known pairs ranged from 0.0 to 0.187. For seven of the 11 known pairs, we were able to reject the hypothesis of a half-sibling relationship in favor of a hypothesis of no genetic relationship. Although the maximum likelihood analysis could not reject a half-sibling relationship for the remaining four pairs, the relatedness values were, in all

cases, lower than expected for such a relationship (expected $R = 0.25$). The possible pairs had relatedness values ranging from 0.0 to 0.169, with a half-sibling relationship rejected in favor of a hypothesis of no genetic relationship in 14 of 17 pairwise comparisons.

Five pairs of individuals from the general population were found to have relatedness values of 1.0. Based on the short period of time between the individuals' captures (average period of time: 5.2 days, median: 1 day) and the absence of any recorded signs of recent wing biopsies on the individuals' left wings in the field notes, it is not likely that these pairs of individuals were unnoticed recaptures. Furthermore, the general population contained 31 individual pairings that possessed a high R value (> 0.36) and 14 of these pairings possessed R values greater than 0.4. Likelihood ratio tests in which a full-sibling relationship (the putative hypothesis) was compared to a half-sibling relationship (the alternative hypothesis) were performed for these 31 pairs. For four of these pairings, all of which had R values greater than 0.4, we rejected the half-sibling relationship. However, these four pairs may represent parent-offspring relationships (expected $R = 0.5$) or full-sibling relationships (expected $R = 0.5$) since the amount of shared genetic information is expected to be the same.

Discussion

We find that the documented behavioral interactions do not appear to be occurring between close relatives. This conclusion is justified by the low R values of the individuals involved in the known and possible pairs, as well as most pairwise likelihood ratio tests rejecting a half-sibling relationship in favor of no genetic relationship. The average R value of the general migrating population mostly supports a pattern of numerous unrelated individuals migrating through this area around the same time. Moreover, the Mann-Whitney U test determined that the behaviorally interacting individuals were not significantly more related than the general population. These results suggest that this area serves as a migration route that may be commonly used due to unknown advantages, such as roost availability, landscape features, or proximity to wintering grounds.

Seven of the 11 known pairs and 14 possible pairs were confirmed to be unrelated based on likelihood ratio tests of low relatedness values. The remaining four known pairs and three possible pairs possessed p values that could not rule out a half-sibling relationship. In addition, the general population contained 31 pairs with high R values that indicated a full-sibling relationship or a parent-offspring relationship. Four of the 31 pairs had p values that rejected a half-sibling relationship, which allows us to conclude that these individuals were either full-siblings or a parent and offspring. Although none of the individuals in these pairs were identified as juveniles or subadults, they were captured later in the year (not earlier than 19 September) and may have been difficult to identify as such. A half-sibling relationship could not be rejected for the remaining 27 pairs, although their R values ranged from 0.361 to 0.5. Nevertheless, this does not remove the possibility of a full-sibling or parent-offspring relationship for these pairs. Overall, these results suggest that migratory behaviors and routes are potentially shared within families even if the members were not observed to directly interact in co-migratory behaviors.

The five unexpected pairs of highly related individuals are also important to note. Since these pairs possess an R value of 1.0 but do not appear to be recaptures, it is likely that these individuals are identical twins especially given the fact that the hoary bat tends to produce twins (Shump and Shump 1982). To our knowledge, this is the first evidence for identical twins in bats. Although it has always been assumed that the hoary bat litter-mates were half-siblings, the

presence of four pairs that had R values between 0.40 and 0.52 and p values that rejected a half-sibling relationship in favor of a full-sibling relationship supports the possibility that the twin pups can be full-siblings or even identical twins. However, further investigations will need to be performed in order to determine the frequency of twinning. Clearly, the reproductive biology of hoary bats is more complex than previously appreciated.

This study has provided important insights into the migratory behaviors and genetic make-up of the hoary bats that pass through the Humboldt Redwoods. The fall migration through this area appears to begin around mid-September, although three hoary bats were netted in early June, and basically ends by the end of November. A large number of migrating hoary bats were netted between late September and early October, suggesting that this is the major time period that this species travels through northwestern California. In addition, the almost complete dominance of males in the sampled population indicates that this area is mostly used by migrating males. This poses some interesting questions about the absence of migrating females that may be travelling to the same wintering grounds. Since females tend to spend the summers in areas farther east than California (Cryan 2003), this study site may have been too far west to sample migrating females. There may be areas further south where the migration routes of the males and females intersect so that mating can occur before the bats enter hibernation.

Acknowledgements

This project was funded by the USDA Forest Service, the Office of Undergraduate Research and Scholarship at GVSU, and the William Schroeder Undergraduate Endowed Fellowship in Chemistry (GVSU). We would like to thank the University of Arizona for performing the fragment analysis of our samples.

References

- Bourke AFG (2014) Hamilton's rule and the causes of social evolution. *Philosophical Transactions of the Royal Society B* 369: 20130362.
- Burland TM, EM Barratt, RA Nichols, PA Racey (2001) Mating patterns, relatedness and the basis of natal philopatry in the brown long-eared bat, *Plecotus auritus*. *Molecular Ecology* 10: 1309-1321.
- Carter GG and GS Wilkinson (2013) Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proceedings of the Royal Society B* 280: e20122573.
- Colbeck GJ, P Duchesne, LD Postma, V Lesage, MO Hammill, J Turgeon (2013) Groups of related belugas (*Delphinapterus leucas*) travel together during their seasonal migrations in and around Hudson Bay. *Proceedings of the Royal Society B* 280: e20122552.
- Cryan PM (2003) Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. *Journal of Mammalogy* 84: 579-593.
- Díaz-Muñoz SL, EH DuVal, AH Krakauer, EA Lacey (2014) Cooperating to compete: altruism, sexual selection and causes of male reproductive cooperation. *Animal Behaviour* 88: 67-78.
- Dingle H and VA Drake (2007) What is migration? *BioScience* 57: 113-121.
- Fleming TH and P Eby (2003) Ecology of bat migration. In: *Bat Ecology* (eds. Kunz TH, Fenton MB), pp. 156-208. University of Chicago Press, Chicago.

- Hamilton WD (1964) The genetical evolution of social behavior. *Journal of Theoretical Biology* 7:1-16.
- Kalinowski ST, AP Wagner, ML Taper (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* 6: 576-579.
- Kerth G, K Safi, B König (2002) Mean colony relatedness is a poor predictor of colony structure and female philopatry in the communally breeding Bechstein's bat (*Myotis bechsteinii*). *Behavioral Ecology and Sociobiology* 52: 203-210.
- Krakauer AH (2005) Kin selection and cooperative courtship in wild turkeys. *Nature* 434: 69-72.
- Krauel JJ and GF McCracken (2013) Recent advances in bat migration research. In: *Bat Evolution, Ecology, and Conservation* (eds. Adams RA, Pedersen SC), pp. 293-313. Springer Science+Business Media, New York.
- Kunz TH, R Hodgkinson and CD Weise (2009) Methods of capturing and handling bats. In: *Ecological and behavioral methods for the study of bats, second edition* (eds Kunz TH, Parsons S), pp. 3-35. Johns Hopkins University Press, Baltimore.
- Moussy C, DJ Hosken, F Mathews, GC Smith, JN Aegerter, S Bearhop (2013) Migration and dispersal patterns of bats and their influence on genetic structure. *Mammal Review* 43: 183-195.
- Oyler-McCance SJ and JA Fike (2011) Characterization of small microsatellite loci isolated in endangered Indiana bat (*Myotis sodalis*) for use in non-invasive sampling. *Conservation Genetics Resources* 3:243-245.
- Piaggio AJ, KEG Miller, MD Matocq, SL Perkins (2009) Eight polymorphic microsatellite loci developed and characterized from Townsend's big-eared bat, *Corynorhinus townsendii*. *Molecular Ecology Resources* 9: 258-260.
- Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103-106.
- Shump KA and AU Shump (1982) *Lasiurus cinereus*. *Mammalian Species* 185: 1-5.
- Trujillo RG and SK Amelon (2009) Development of microsatellite markers in *Myotis sodalis* and cross-species amplification in *M. grisescens*, *M. leibii*, *M. lucifugus*, and *M. septentrionalis*. *Conservation Genetics* 10:1965-1968.
- Van Oosterhout C, WF Hutchinson, DPM Wills, P Shipley (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535-538.
- Weller T and S Giordano (2013) Echolocation behavior of hoary bats during migration provides insights into their disproportionate fatality rate at wind energy facilities. International Bat Research Conference, San Jose, Costa Rica.
- Worthington Wilmer J and E Barratt (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News* 37: 1-3.