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D'Urban Jackson, J., Dos Remedios, N., Maher, K.H. et al. (8 more authors) (2017)
Polygamy slows down population divergence in shorebirds. *Evolution*. ISSN 0014-3820

<https://doi.org/10.1111/evo.13212>

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Polygamy slows down population divergence in shorebirds

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/evo.13212](https://doi.org/10.1111/evo.13212).

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Running head: Mating systems and diversification in shorebirds

Keywords: Mating systems, sexual selection, dispersal, shorebird, gene flow, speciation, migration

Data archival location: doi:10.5061/dryad.vn77k

Acknowledgments

We thank F. Hailer and two anonymous reviewers for their suggestions which improved the manuscript. This work was funded by a NERC GW4+ studentship NE/L002434/1 awarded to JDJ. We thank numerous fieldworkers and collaborators who helped to collect or provided DNA samples of the datasets used. Additional microsatellite genotyping of previously unpublished data sets was done at NBAF-Sheffield supported by grants (NBAF547, NBAF933, NBAF441). TS was supported by Hungarian Science Foundation (NKFIH-2558-1/2015) and was a Fellow at the Advanced Institute of Berlin. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors declare no conflicts of interest.

Author contributions: C.K. and T.S. designed the study; C.K., N.d.R, K.H.M., S.Z. S.O-M., S.H., D.B. and T.B. provided samples and/or microsatellite genotypes. C.K. and J.D.J. discussed the analysis methods and J.D.J. performed the analysis. J.D.J., C.K., M.W.B., and T.S wrote the first draft and all authors contributed the final manuscript.

Abstract

Sexual selection may act as a promotor of speciation since divergent mate choice and competition for mates can rapidly lead to reproductive isolation. Alternatively, sexual selection may also retard speciation since polygamous individuals can access additional mates by increased breeding dispersal. High breeding dispersal should hence increase gene flow and reduce diversification in polygamous species. Here we test how polygamy predicts diversification in shorebirds using genetic differentiation and subspecies richness as proxies for population divergence. Examining microsatellite data from 79 populations in ten plover species (Genus: *Charadrius*) we found that polygamous species display significantly less genetic structure and weaker isolation-by-distance effects than monogamous species. Consistent with this result, a comparative analysis including 136 shorebird species showed significantly fewer subspecies for polygamous than for monogamous species. By contrast, migratory behaviour neither predicted genetic differentiation nor subspecies richness. Taken together, our results suggest that dispersal associated with polygamy may facilitate gene flow and limit population divergence. Therefore, intense sexual selection, as occurs in polygamous species, may act as a brake rather than an engine of speciation in shorebirds. We discuss alternative explanations for these results and call for further studies to understand the relationships between sexual selection, dispersal and diversification.

Introduction

Sexual selection is often thought of as a facilitator of speciation via female mate preferences leading to prezygotic reproductive isolation (the “engine-of-speciation” hypothesis; Morrow

et al., 2003). Intense sexual selection can lead to rapid speciation in at least four different ways (Ritchie, 2007; Wilkinson and Birge, 2010; Gavrillets, 2014). First, female preference for males that exhibit particular traits may lead to coevolution between males exhibiting the traits and females preferring the trait either via selection for good genes or sexy sons (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982; West-Eberhard, 1983; Fowler-Finn and Rodríguez, 2016; Ellis and Oakley, 2016). Second, negative frequency dependent selection on sexually selected traits that are important during intrasexual competition may ultimately result in reproductive isolation. (Greene et al., 2000; Seehausen and Schluter, 2004; Clutton-Brock and Huchard, 2013). Third, sexual selection might be associated with ecological speciation where sexually selected traits or those involved in sexual communication are under divergent natural selection (Maan and Seehausen, 2011; Safran et al., 2013). Fourth, sexually antagonistic coevolution, termed sexual conflict (Parker, 1979), between males and females may drive an arms race with male and female (counter) adaptations that lead to exaggerated traits which then form reproductive barriers (Gavrillets, 2014).

By contrast, sexual selection may also reduce – instead of amplify - reproductive isolation between populations under some evolutionary scenarios. For example, sexual conflict may enhance interpopulation gene flow if female resistance to pre- and postmating manipulation prevents matings in some populations, therefore, promoting the dispersal of local males to find naïve females that have not developed counteradaptations in neighbouring populations (Parker and Partridge 1998). In addition, sexual selection could also limit sympatric speciation as assortative mating can reduce the variation that could be selected upon i.e. the fixation of certain traits (Kirkpatrick and Nuismer, 2004).

Variance in mating success is typically larger in polygamous than in monogamous species. Polygamous individuals attempt to access as many mates as possible and may need to disperse, especially when breeding is highly synchronised locally, to maximise their reproductive success. Dispersal to increase mate access has been suggested to explain why adults of polygamous and promiscuous birds and mammals travel large distances during the breeding season (Blundell et al., 2002; Woolfenden et al., 2005; Debeffe et al., 2014; García-Navas et al., 2015, Davidian et al., 2016; Kempenaers and Valcu, 2017), whereas monogamous species are often more faithful to breeding sites (Pitelka et al., 1974; Saalfeld and Lanctot, 2015). High breeding dispersal is likely to lead to low levels of genetic differentiation within a polygamous species (Küpper et al., 2012; Verkuil et al., 2012; Eberhart-Phillips et al., 2015). This gene flow may prevent reproductive isolation by counteracting the effect of processes such as genetic drift and local adaptation and thus slow speciation processes (here termed the “dispersal-to-mate” hypothesis).

Regular migration movements outside the breeding season may also influence diversification (Phillimore et al., 2006; Garant et al., 2007; Weeks and Claramunt, 2014; Arendt, 2015). Intuitively, high dispersal abilities should reduce genetic differentiation between populations (Belliure et al., 2000; Garant et al., 2007; Claramunt et al., 2012; Weeks and Claramunt, 2014). Indeed, many examples of low genetic differentiation among breeding populations of migratory species are found in birds and mammals (e.g. Webster et al., 2002; Friesen et al., 2007; Burns and Broders, 2014). However, high (and leptokurtic) dispersal can also lead to the colonisation of remote areas such as oceanic islands that are too far away from the core

population to maintain regular gene flow. After the colonization event, local adaptation and genetic drift in combination with behavioural changes may then lead to allopatric differentiation (Rosenzweig, 1995; Owens et al., 1999; Phillimore et al., 2006). Corroborating this hypothesis, seasonal migration has been linked to greater net diversification rates in birds where colonisation events are followed by settling down and loss of annual migratory behaviour (Rolland et al., 2014).

Shorebirds (sandpipers, plovers and allies; *Scolopaci*, *Thinocori*, *Chionidi* and *Charadrii*) are a diverse and ecologically well-characterised avian clade which display huge variation in mating systems and migratory behaviour (Székely et al., 2000; Piersma and Lindström, 2004; Thomas et al., 2007; García-Peña et al., 2009). This group of taxa therefore provide an ideal opportunity to investigate the relationship between mating systems, migratory behaviour and diversification. The objectives of our study were to test whether polygamous species that are under high pressure to access multiple mates, and thus are subject to strong sexual selection, showed higher diversification than monogamous species, as predicted by the “engine-of-speciation” hypothesis or lower diversification consistent with the “dispersal-to-mate” hypothesis. Mating systems have a significant influence on the variation of individual mate success, with polygamy leading to greater variation in mating success across individuals compared to monogamy (Emlen and Oring, 1977, Shuster and Wade, 2003). For this reason we used mating system as a proxy for strength of sexual selection as we hypothesised that due to this high variation in breeding success, polygamous individuals move between breeding populations in an attempt to elevate their chance of successful mating (Breiehagen, 1989; Székely and Lessells, 1993; Stenzel et al., 1994; Kempenaers and Valcu, 2017).

We investigated the relationships between mating systems, migration and diversification using two data sets with either genetic differentiation or subspecies richness as proxy for within species population divergence and hence speciation propensity. Firstly, we studied plovers (*Charadrius* spp) – a globally distributed clade of shorebirds that includes both migrant and resident species with monogamous or sequentially polygamous mating systems (Thomas et al., 2007; dos Remedios et al., 2015). Within a breeding season sequentially polygamous plovers change partners after a successful breeding attempt leaving their mate to care for the young, whereas, monogamous plovers stay together for subsequent breeding attempts. Social mating system reflects genetic mating system in plovers, since extra-pair paternity is rare in these species (less than 5 %, Maher et al., provisionally accepted). Using ten *Charadrius* species we tested whether intraspecific patterns of genetic differentiation were associated with mating system and/or migratory behaviour using microsatellite datasets. Secondly, since similar genetic data are only available for a fraction of shorebirds we expanded our analyses to include 136 shorebird species and test whether mating system and/or migratory behaviour predicted subspecies richness, an alternative measure for diversification (Belluire et al., 2000; Phillimore and Owens, 2006, Martin and Tewksbury, 2008).

Materials and methods

Genetic differentiation in plover populations

We analysed published and newly collected microsatellite data from ten plover species (Genus: *Charadrius*): Kittlitz's plover (*C. pecuarius*; Eberhart-Phillips et al., 2015; dos Remedios, 2013), Madagascar plover (*C. thoracicus*; Eberhart-Phillips et al., 2015), white-fronted plover (*C. marginatus*; Eberhart-Phillips et al., 2015 and dos Remedios, 2013), chestnut-banded plover (*C. pallidus*; dos Remedios 2013), Kentish plover (*C. alexandrinus*; Küpper et al., 2012), mountain plover (*C. montanus*; Oyler-McCance et al., 2008) and piping plover (*C. melodus*; Miller et al., 2010). In addition, further plover populations from three species were genotyped including, snowy plover (*C. nivosus*), common ringed plover (*C. hiaticula*) and killdeer (*C. vociferous*). Sampling locations were distributed across all continents except Australia, South America and Antarctica (Table 1; Figure 1.) and included four resident and six migratory species with different mating systems (six monogamous and four polygamous) and wide variation in breeding range sizes (Table 1). The detection of spatial genetic pattern can be highly sensitive to factors such as the number of loci and the number of alleles per locus (Landguth et al., 2012), however, across the datasets we found no relationship between the number of loci or the average number of alleles per locus and the detection of spatial genetic patterns (see Supplementary material). For microsatellite marker characteristics and laboratory protocols see supplementary material Table S1.

Due to potential bias of null alleles during the estimation of population subdivision (F_{ST}) and genetic distance (Chapuis and Estoup, 2007; Dabrowski et al., 2014), null allele frequencies and genotyping errors were estimated for all data using Microchecker 2.2.3 (Van Oosterhout et al., 2004). Loci identified as having null alleles in the majority of the populations were

removed for Bayesian clustering analysis, and pairwise F_{ST} values were corrected for the presence of null alleles using FreeNA (Chapius and Estoup, 2007). Individuals with more than 15% missing data were excluded from further analyses.

We used a Bayesian clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to determine the extent of population structure within each species. We used the admixture model with location information as a prior, an approach that is required when structure is expected to be weak (Pritchard et al., 2000; Hubisz et al., 2009). This approach improves cluster outcomes by favouring the clustering of individuals that were sampled together. However, it is worth noting that this method does not detect structure if there is none (Pritchard et al., 2000; Hubisz et al., 2009). Location priors for each population are provided in Table 1. For breeding locations with less than 10 samples we ran the analysis twice, first giving them unique location priors and again after removing these populations. All analyses were run with a burn-in period of 100,000 followed by 1,000,000 Monte Carlo Markov Chain (MCMC) repeats for ten replicates. Initially, the number of clusters tested were between one and the maximum number of locations sampled (Table 1). We then summarised the results with STRUCTURE HARVESTER v 0.6.94 (Earl and VonHoldt, 2012) and estimated the most likely number of clusters present based on likelihood and Delta K (Evanno et al., 2005). Bar plots representing admixture proportions for the most likely K values were examined to assess whether the results of Delta K and likelihood methods were biologically meaningful. Individual admixture proportion information was merged from the ten repeats using the “full search” method in CLUMPP v 1.1.2 (Jakobsson and Rosenberg, 2007). If the initial best model suggested $K \geq 2$ and the admixture proportions of individuals

within these populations was less than 0.01, the data set was split into the identified clusters and we repeated the Bayesian clustering until the best model in STRUCTURE was $K = 1$, similar to progressive partitioning (Hobbs et al., 2011).

The number of clusters identified by STRUCTURE were compared for species with different mating systems ('monogamous' or 'polygamous') and migratory behaviour ('resident' or 'migratory'). Sea distance is an effective barrier of gene flow in plovers (Küpper et al., 2012). For species distributed and sampled on more than one land mass, we included only the data set with the largest number of samples and locations. Species were assigned to categories 'one' or 'more than one genetic clusters' and compared frequencies to expected 1:1 values using Fisher's exact tests (Fisher, 1922).

We used the scoring system based on Thomas et al., (2007) to classify the mating system of each species (Székely et al., 2004; García-Peña et al., 2009; Olson et al., 2009) and updated the mating system information for species with new data (Supplementary material Table S2 and S3). However, we simplified the scoring for the purpose of this study using only two instead of five categories, since only a few dispersers per generation are required to maintain gene flow (Spieth, 1974; Mills and Allendorf, 1996). We classified the categories 0 and 1 of Thomas et al., (2007), which correspond to $\leq 1\%$ polygamy in either sex observed during breeding behaviour studies, as 'monogamous' and groups 2-4 (for species that are known to display more than 1 % polygamy in either sex) as 'polygamous'. Migratory status was classified as either 'migrant' (including partially migrant species) or resident. Migratory

information was collected from Bird Life International (<http://www.birdlife.org/datazone/species>, accessed: January 2017) (Supplementary material Tables S2 and S3).

To examine the degree of isolation by distance (IBD) for the ten plover species we performed Mantel Tests (Mantel, 1967; Mantel and Valand, 1970) using a population based approach instead of alternative landscape genetic approaches (e.g. multiple regression analysis) since individual location and environmental data were not available for all species. We calculated Euclidean distance matrices between populations using GenALEX 6.501 (Peakall and Smouse, 2012). Using log-transformed geographic distances (Legendre and Fortin 2010) provided the same qualitative results (results not shown). We calculated pairwise Rousset's linearised F_{ST} (' F_{ST} ' hereafter) from the null allele corrected F_{ST} values, using the following equation: $F_{ST} / (1 - F_{ST})$ (Rousset, 1997). All Mantel tests were performed with the package 'adegenet' (Jombart, 2008).

To test whether mating system and migratory status effects spatial genetic patterns, we used the slope of a linear regression line between genetic (F_{ST}) and geographic distance for each species as a proxy for the strength of IBD ('IBD gradient' hereafter). This was calculated because of potential bias involved in directly comparing average F_{ST} values between species due to the ascertainment biases of microsatellite markers, since 75% of the markers used were developed for one species specifically (Küpper et al., 2007).

Following tests for normality of the IBD gradient, we performed phylogenetic least squares analysis (PGLS; Freckleton et al., 2002) to account for phylogenetic autocorrelation between species using the ‘caper’ package (Orme, 2013) in addition to generalised linear models (GLM) with Gaussian errors to examine the influence of mating system and migratory behaviour on the IBD gradient using ‘species’ as the statistical unit. The recently published *Charadrius* phylogeny (dos Remedios et al., 2015) was used to measure phylogenetic relatedness between species for the PGLS analysis. Species with large breeding range sizes are likely to have greater levels of differentiation between populations compared to those with smaller ranges (Gavrilets and Vose, 2005; Losos and Parent, 2009; Kisel and Barraclough, 2010), therefore we incorporated breeding range size into the models. Due to large differences between species breeding range sizes, which may influence the IBD gradient, log breeding range size was included in the model. As our sample size is small ($n=10$) we fitted and compared single parameter models to avoid overfitting of models that may lead to inflation of statistical significance (Harrell, 2001). The best fitting model was selected using an information theoretic approach (Burnham and Anderson, 2002). This method ranks the models based on Akaike information criterion corrected for small sample sizes (AICc) and we assessed support for each model based on the differences in AICc (Δ_i) and Akaike weights (w_i) (Burnham and Anderson, 2002). Substantial support for a model is indicated by Δ_i - values of less than 2 and of these, highly optimal models will have w_i values of more than 0.9 (Burnham and Anderson, 2002). Model selection was performed using the ‘MuMIn’ package (Bartoń, 2016).

Subspecies richness in shorebirds

To test our hypotheses that 1) polygamy restricts diversification and 2) migration restricts diversification, we used the subspecies richness of shorebird species (Order: *Charadriidae*; suborders: *Charadrii*, *Chionidi*, *Scolopaci* and *Thinocori*) as a proxy for the degree of diversification. This allowed us to test for drivers of diversification in a much larger data set. Avian subspecies richness has been used as a proxy for population differentiation in previous studies testing the drivers of diversification (Belliere et al., 2000; Phillimore and Owens, 2006, Martin and Tewksbury, 2008). We used subspecies information from the IOC World Bird List v 7.1 (Gill and Donsker, 2016). This database is updated annually with new information from peer reviewed articles. Subspecies delineations are not always supported by genetic data (Phillimore and Owens, 2006), however, in absence of genetic data these delineations provide a useful proxy for diversification in comparative studies at lower taxonomic levels. We classified mating systems and migratory status as the same used for the plover analyses above (Supplementary material Tables S2 and S3). We again performed PGLS analysis and in addition to mating system and migratory status we also included log breeding range size. Shorebirds without mating system information or with only anecdotal evidence of mating system category were excluded, as were species without breeding range size data.

We selected 100 phylogenetic trees at random using the phylogeny of Jetz et al., (2012), downloaded from <http://birdtree.org> (accessed in: December 2016). We repeated the analysis using both Hackett et al., (2008) and Ericson et al., (2006) phylogenetic backbones and no differences were found between the methods. We removed four species (*C. nivosus*,

Coenocorypha huegeli, *Nycticryphes semicollaris* and *Gallinago delicata*) from the analysis as they were not included in the Jetz et al., (2012) phylogeny. This resulted in a final dataset of 136 shorebirds species (Supplementary material Tables S2 and S3) consisting of 109 monogamous species, 27 polygamous species or 83 migrant species and 53 resident species.

PGLS analysis was repeated for each of the 100 trees and the original model formula was as follows:

Total number of subspecies \sim mating system + migratory status + migratory status * mating system + log₁₀ breeding range size

We then simplified models removing the least significant variable in a stepwise manner. As with IBD gradient GLMs we assessed the model fit for all model combinations using Δ_i and w_i values (Burnham and Anderson, 2002).

For all statistical analyses, unless stated otherwise, we used R version 3.3.2 (R Development Core Team, 2016).

Results

Genetic differentiation in plovers

We identified one locus, Calx14 with a high probability of having null alleles in the killdeer, this locus was excluded from further analyses in this species. The average number of alleles per locus indicated large variation in genetic diversity between species (mean = 6.4 ± 3.5 SD). No difference in the clustering outcome was found when removing populations with less than ten individuals (data available on request). Progressive partitioning increased piping plover clustering outcome from two to three, indicating that in addition to a split between the two subspecies (*C. m. circuminctus* and *C. m. melodus*), there is also a differentiation in *C. m. melodus* between the Canadian and U.S. American sampling sites (Figure 2(b)). Mating system but not migratory behaviour was associated with the number of genetic clusters across the ten species (Fisher's exact tests: mating system: $p = 0.033$; migratory status: $p = 1$). We found fewer clusters within polygamous (mean \pm SD: 1.25 ± 0.5) than within monogamous species (2.33 ± 0.5). We did not detect any differentiation within three of the four polygamous species across their sampled breeding populations (Figure 2A), whereas we detected at least two genetic clusters within all six monogamous species, comprising two clusters in four species and more than two clusters in two species (Figure 2B). The white-fronted and Kittlitz's plover exhibited consistent patterns between Madagascar and the African mainland, i.e. we detected genetic structure among monogamous white-fronted plover populations but not among polygamous Kittlitz's plover populations within each land mass. To avoid pseudoreplication we included only the larger Madagascar data set for both species in the subsequent analyses.

Across all plovers IBD was weak (Figure 3 and Table 2). Three monogamous species, white-fronted plover, piping plover and the common ringed plover showed significant IBD (Monte

Carlo test observation, $r = 0.397, 0.749$ and 0.28 respectively; $p = 0.05, 0.02, 0.05$ respectively; Table 2), whereas for all other species we did not detect a significant association. The best model to explain variation in IBD gradient among the ten plover species contained only ‘mating system’ as an explanatory variable (PGLS $w_i = 0.86$) and no other model had a $\Delta_i \leq 2$. The model suggested that monogamous species have significantly higher IBD gradients than polygamous species (PGLS: $df = 8, t = -2.49, p = 0.05$). Neither breeding range size nor migratory status predicted IBD gradients in plovers. For full model results of the PGLS and the GLM analyses see supplementary material Table S4.

Subspecies richness in shorebirds

Phylogenetic analysis in shorebirds showed that subspecies richness was best predicted by a model that included both mating system and breeding size range (Supplementary material Table S6). The minimal model indicated that monogamous species are divided into significantly more subspecies than polygamous species (Figure 4) and shorebirds with larger breeding ranges harboured more subspecies than small range species (PGLS model 3: $df = 133$ mating system $t = -2.26, p = 0.026$; log breeding range size $t = 1.98, p = 0.05$). Consistent with genetic results in plovers, migratory behaviour did not predict subspecies richness (PGLS model 2: $df = 132$, migratory behaviour $t = -0.165, p = 0.896$; Supplementary material Table S5).

Discussion

We investigated whether diversification in shorebirds is related to mating and/or migration behaviour using two complementary indices of population diversification: genetic differentiation in *Charadrius* plovers and subspecies richness across shorebird species. Consistent with previous studies (Møller and Cuervo, 1998; Owens et al., 1999; Arnqvist et al., 2000) we found a relationship between mating system and diversification. However, contrary to previous suggestions that sexual selection facilitates speciation (West-Eberhard, 1983; Panhuis et al., 2001; Ritchie, 2007) we found that polygamous shorebird species (i.e., those with higher competition for mates), showed less genetic structure, weaker isolation-by-distance and lower subspecies richness compared to monogamous species. These results are consistent with the “dispersal-to-mate” hypothesis (i.e. intense sexual selection in polygamous species promotes breeding dispersal), which in turn leads to widespread gene flow across the distribution range (Küpper et al., 2012). Our interpretations are supported by recent direct studies on breeding dispersal of polygamous sandpipers using satellite tag telemetry, where lekking male pectoral sandpipers show exceptional long distance breeding dispersal moving more than 13,000 km during a single breeding season in search for new mating opportunities (Kempnaers and Valcu, 2017). Similarly, in polygynous mammals polygynous males disperse between neighbouring populations, presumably to increase their access to oestrus females (Greenwood, 1980, Olupot and Waser 2001) suggesting that the dispersal of the polygamous sex is influenced by the distribution of the opposite sex (Greenwood, 1980).

Using genetic data from multiple shorebirds we show the evolutionary consequences of mating behaviour at the population level. Instead of promoting genetic isolation of populations, sexual selection rather seems to constrain speciation due to mate access pressure. The results on the genetic differentiation of plover populations were mirrored by our findings of subspecies richness across shorebirds that showed fewer subspecies in polygamous compared to monogamous shorebird species. Both data sets included polygynous and polyandrous taxa and hence sequentially polygamous males and females may be responsible for maintaining high gene flow. Subspecies delineations are based often largely on divergent phenotypic characters and do not necessarily reflect findings on differentiation of neutral genetic markers (Phillimore and Owens, 2006). Nevertheless, in our study, we found genetic support for all subspecies delineations within the plover species analysed (Supplementary material table S6). Further, since subspecies definitions vary widely among authors and may not be supported by genetic data, subspecific delineation may in any case provide a complementary measure of ecological divergence that is then also associated with mating systems. Finally, subspecies richness may represent a more conservative measure for population differentiation than genetic differentiation since we found additional genetic structure *within* subspecies in the piping plover (*C. m. melodus*, Figure 2(b)), the common ringed plover (*C. h. hiaticula* and *C. h. tundra*, Figure 2(a)) and the snowy plover (*C. n. nivosus*, Figure 2 (a)).

Our findings contribute to the debate concerning the role of sexual selection in speciation (Gage et al., 2002; Morrow et al., 2003; Kirkpatrick and Nuismer, 2004; Maan and Seehausen, 2011; Servedio and Kopp, 2012; Servedio and Bürger, 2014; Ellis and Oakley,

2016). Previous studies have suggested at least five arguments to explain why sexual selection may not appear to promote diversification. Firstly, inconsistent results may emerge if both speciation and extinction rates are elevated in sexually selected species, and these two processes counterbalance each other (Morrow et al., 2003, but see: Morrow and Fricke, 2004). Secondly, different mating systems may evolve between species after speciation has occurred (e.g., some clades may be more likely to develop certain breeding behaviour than others) and thus sexual selection is independent of speciation due to other mechanisms (e.g., local adaptation (Gage et al., 2002)). Thirdly, sexual selection may play a part in speciation, but mate preference alone may not be strong enough to prompt complete reproductive isolation (van Doorn et al., 2004; Servedio, 2011; Servedio and Kopp, 2012; Servedio and Bürger, 2014). Fourthly, the effects of ecological speciation may mask the influence of sexual selection and these two forces could work antagonistically or together in speciation processes (Kraaijeveld et al., 2011; Maan and Seehausen 2011; Wagner et al., 2012). Finally, these inconsistent findings may in part be due to differences in methodologies used to investigate the relationship between sexual selection and speciation (Kraaijeveld et al., 2011).

Here we provide a hypothesis which emphasises that dispersal driven by mate access pressure needs to be taken into account in discussions concerning the importance of sexual selection in diversification processes. According to the “dispersal-to-mate” hypothesis, polygamous adults (polyandrous females or polygynous males) looking for new mates may often disperse to increase their pool of potential mates. When polygamous individuals reproduce at several sites they become a major contributor to high gene flow. Field data suggest that male polygamous sandpipers disperse large distances during the breeding season (Kempnaers and

Valcu, 2017) and similarly, female polyandrous plovers tend to exhibit larger scale movements than males (Székely and Lessells 1993; Stenzel et al., 1994). These differences will ultimately be reflected in population genetic patterns. Consistent with female biased dispersal, maternally inherited mtDNA is less structured, whereas the Z-chromosomal DNA is more structured than autosomal microsatellites in the polyandrous Kentish plover (Küpper et al., 2012). However, the latter result may also reflect typical sex-specific natal dispersal patterns where female birds disperse more than males (Greenwood, 1980; but see: Mabry et al., 2013).

Natal dispersal may chiefly serve to avoid inbreeding but it has been also been linked to the mating system (Greenwood 1980). Sex-biased dispersal in birds and mammals may be related to either resource defence (birds) or mate defence (mammals) and hence related to mating strategies. Greenwood (1980) suggested that avian monogamy is consistent with a resource defence mating system which leads to female biased dispersal, whereas polygamy is linked to mammalian mate defence and male biased dispersal. In contrast to natal dispersal, our results imply that breeding dispersal will be dictated by the direction of polygamy, i.e. female biased in polyandrous population but male biased under polygyny. Two processes may explain why polygamous species have lower population divergence levels compared to monogamous species. In species with high sexual selection such as lekking species, males may either disperse to compete for additional mates, exploiting locally synchronised females (Kempnaers and Valcu, 2017) or in the case of subordinate males they may disperse to find a space on a lek (Greenwood, 1980). Habitat and mate availability may also be a strong factor

driving female breeding dispersal in polyandrous species (Küpper et al. 2012, Cruz-Lopez et al., accepted).

In this study we are unable to determine the relative influence of natal versus breeding dispersal. To distinguish between the influence of natal and breeding dispersal on spatial genetic patterns, in addition to establishing whether dispersal patterns do truly differ between monogamous and polygamous species as predicted by the “dispersal-to-mate” hypothesis, further genetic, direct tracking and ringing studies are necessary. For example, a direct comparison of dispersal propensity between males and females within species representing different mating systems would provide strong evidence to support or refute the “dispersal-to-mate” hypothesis. Despite huge recent technological advances in direct tracking (Kays et al. 2015), methodological challenges such as the weight of tags have so far constrained our ability to compare detailed movement behaviour across an equivalent group of species as used in this study.

Contrary to our predictions, we found no support that annual migration influences spatial genetic patterns or subspecies richness in shorebirds. By undertaking seasonal migration, one would predict that migratory species have a higher dispersal ability than resident species and that this may promote higher gene flow between breeding populations (Winker, 2000; Claramunt et al., 2012; Weeks and Claramunt, 2014). A possible reason for this is that migratory species may vary in their degree of migratory connectivity. Migratory connectivity is the strength of the association between a breeding site and a wintering site, i.e. strong

migratory connectivity is when individuals from one breeding ground always migrate to the same wintering ground, whereas weak migratory connectivity reflects the mixing of populations on both breeding and wintering grounds (reviewed in: Webster et al., 2002). Strong connectivity between breeding and wintering grounds can result in genetic divergence between populations (Rundel et al., 2013), however, the degree of connectivity is highly variable between and even within species (Rundel et al., 2013, Webster et al., 2002). Therefore, the presence or absence of genetic structure and variable IBD gradients within the six migrant plover species in our plover dataset as well as the variation in subspecies richness of migratory shorebirds, may reflect different levels of migratory connectivity between species. In addition, the migratory category of this study encompasses species which vary in different aspects of migration such as distance travelled, the proportion of the population migrating and wintering habitat, all of which could have implications for breeding site genetic structure and by proxy, subspecies richness. For example, Kraaijeveld (2008) found support for habitat stability affecting subspecies richness in shorebirds with species that overwinter at unstable inland wetlands showing lower subspecies numbers than those overwintering at coastal sites, which are characterized by more stable conditions. Habitat stability might also shape patterns of breeding dispersal with plovers breeding in volatile habitats being more likely to disperse than those breeding under stable conditions. Nevertheless, a higher propensity for dispersal might enable species to reach remote, isolated locations such as oceanic islands where they subsequently evolve into new species in allopatry (Phillimore et al., 2006). The exact use of species and subspecies delineation in avian taxonomy is currently debated with disagreement about which species concept(s) are the easiest to operationalise (Sangster 2014, Barrowclough et al. 2016) and concerns about inappropriate grouping of populations (Gill 2014). We therefore decided to focus our

analyses on continental species distributions only and hence were not able to evaluate this hypothesis in shorebirds.

Present day spatial genetic patterns are the result of a multitude of past and present factors including demographic history (Excoffier, 2004), habitat connectivity (Epps and Keyghobadi, 2015) and range size (Phillimore et al., 2006). Although we did find that higher subspecies richness was associated with larger range sizes, supporting previous work (e.g. Salisbury et al., 2012), there was no such association within the plover data set. This is particularly interesting as two of the four polygamous species, Kentish and Kittlitz's plover, have extremely large breeding range sizes estimated at 13.6M km² and 16.4M km² (<http://www.birdlife.org/datazone/species>; accessed in: January 2017) respectively, yet both exhibit a distinct lack of continental genetic differentiation (see: Küpper et al., 2012 and dos Remedios, 2013), although their island populations are genetically differentiated.

Future studies are essential to further investigate the relationships between sexual selection, mate choice and breeding dispersal. New studies are needed to de-couple natal and breeding sex-biased dispersal patterns and to compare these across species representing different mating systems. To test the broader relevance of the “dispersal-to-mate” hypothesis it is necessary to explore the theoretical basis of how selection for high mate access promotes dispersal and the population genetic consequences of this movement. Theoretical studies have been conducted to explain sex-biased dispersal in relation to mating systems (e.g. Kokko and

Rankin, 2006; Shaw and Kokko, 2014), and these models provide excellent starting points for analysing mate access pressure, dispersal and gene flow in relation to sexual selection.

In conclusion, we found that polygamous shorebirds exhibit reduced genetic differentiation compared to monogamous ones, consistently with a previous study carried out on Malagasy plovers (Eberhart-Phillips et al., 2015). These results oppose the notion that sexual selection promotes diversification per se. On the contrary, it appears that polygamy – usually associated with intense sexual selection – inhibits diversification in shorebirds by promoting gene flow among distant continental sites. Future studies are needed to test the robustness of this hypothesis in other taxa with variation in mating systems.

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Tables

Table 1. Summary of sample characteristics for plover species and populations included in genetic differentiation analyses. Mating system references are provided in Table S3.

Information on breeding range size, mating system and migratory status are provided at species level. White-fronted and Kittlitz’s plover mainland Africa populations were used only to corroborate spatial patterns found on Madagascar where sampling was more fine scale.

Breeding range size is not provided for mainland Africa white-fronted and Kittlitz’s plover populations. Loc Prior = different letters correspond to different location prior groupings.

Species	Subspecies	Population (Map number Loc Prior)	Latitude, longitude	N	Breeding range size (Km ²)	Mating system	Migratory status
piping plover	<i>circumcinctus</i>	Prairie North (1 A)	53.2, -110.8	6			
<i>Charadrius melodus</i>	<i>circumcinctus</i>	Prairie South (2 B)	51.4, -106.0	1 8			
Miller et al. 2010	<i>circumcinctus</i>	Great Plains North (3 C)	47.6, -102.1	2 4	221,000	Monogamous	Migratory
	<i>circumcinctus</i>	Great Plains South (4 D)	42.8, -97.4	2 3			
	<i>circumcinctus</i>	Great Lakes (5 E)	45.8, -85.6	1 3			
	<i>melodus</i>	Atlantic Canada (6 F)	45.9, -63.4	6			

				6			
	<i>melodus</i>	Atlantic USA (7 G)	39.6, -73.8	7 0			
mountain plover		Northern (8 A)	47.9, -107.9	2 1			
<i>Charadrius montanus</i>		Central (9 B)	40.8, -104.0	3 4			
Oyler-McCance et al. 2008	N/A				759,000	Polygamous	Migratory
		Montane (10 C)	39.3, -106.0	1 5			
		Southern (11 D)	37.9, -103.1	2 4			
killdeer plover	<i>vociferus</i>	Summer Lake (12 A)	42.8, -120.8	2 4			
<i>Charadrius vociferus</i>	<i>vociferus</i>	Honey Lake (13 B)	40.3, -120.3	2 5	9,100,000	Monogamous	Migratory
(this study)	<i>vociferus</i>	Ceuta (14 C)	23.9, -106.9	2 6			
snowy plover	<i>nivosus</i>	Utah (15 A)	41.2, -112.3	2 5			
<i>Charadrius nivosus</i>	<i>nivosus</i>	San Quintín (16 B)	30.6, -116.0	2 2			
(this study)	<i>nivosus</i>	Florida (17 C)	29.9, -85.5	4 3	1,600,000	Polygamous	Migratory
	<i>nivosus</i>	Ceuta (18 D)	23.9, -106.9	2 5			
	<i>nivosus</i>	Nayarit (19 E)	22.4, -105.6	8			
	<i>nivosus</i>	Texcoco (20 F)	19.5, -99.0	2 3			
common ringed plover	<i>tundrae</i>	Lapland (21 A)	68.4, 18.5	9			
<i>Charadrius hiaticula</i>	<i>tundrae</i>	Varanger (22 B)	70.3, 30.7	1 2			
(this study)	<i>tundrae</i>	Northern Europe (23 C)	67.7, 63.6	7			
	<i>tundrae</i>	Taimyr (24 D)	72.9, 105.9	1 6	4,530,000	Monogamous	Migratory
	<i>tundrae</i>	North east Chukotka (25 E)	67.1, -174.5	1 0			
	<i>tundrae</i>	East central Chukotka (26 F)	64.7, 177.8	1 1			
	<i>tundrae</i>	South east Chukotka (27 G)	62.5, 177.0	2 3			
	<i>hiaticula</i>	S.Sweden (28 H)	57.3, 12.1	2			

				5			
	<i>hiaticula</i>	Belarus (29 I)	52.1, 27.7	1 2			
Kentish plover	<i>alexandrinus</i>	Doñana (30 A)	36.4, -6.4	2 5			
<i>Charadrius alexandrinus</i>	<i>alexandrinus</i>	Fuente de Piedra (31 B)	37.1, -4.8	2 5			
Küpper et al., 2012	<i>alexandrinus</i>	Gharifa (32 C)	35.2, -6.4	1 1			
	<i>alexandrinus</i>	Samouco (33 D)	38.7, -8.9	2 5			
	<i>alexandrinus</i>	Beltringharder Koog (34 E)	54.5, 8.9	1 3			
	<i>alexandrinus</i>	Kujalnik (35 F)	46.8, 30.6	1 5	13,600,000	Polygamous	Migratory
	<i>alexandrinus</i>	Tuzla (36 G)	36.7, 35.1	2 5			
	<i>alexandrinus</i>	Al Wathba (37 H)	24.3, 54.6	2 5			
	<i>alexandrinus</i>	Lake Eton (38 I)	49.1, 46.7	1 4			
	<i>alexandrinus</i>	Xinjiang (39 J)	47.7, 87.5	7			
	<i>alexandrinus</i>	Bohai (40 K)	39.1, 118.2	5			
Kittlitz's plover		Senegal 41 Z)	16.4, -16.3	1 3			
<i>Charadrius pecuarius</i>		Gabon (42 Y)	-0.5, 10.0	8			
dos Remedios, 2013 (African mainland)	N/A	Kenya (43 X)	-0.5, 36.3	2 8		Polygamous	Resident
		Tanzania (44 W)	-2.9, 35.9	2			
Eberhart-Phillips et al., 2015 (Madagascar)		Namibia (45 V)	-18.9, 16.4	2			
		Namakia (54 A)	-15.9, 45.8	2 9	587,000		

		Tsiribihina Delta (56 B)	-19.7, 44.4	4			
		Kirindy Mite (57 C)	-20.9, 43.9	5			
		Fanjakana (58 D)	-21.7, 45.1	3			
		Mangoky (59 E)	-21.7, 43.4	2			
		Morombe (60 E)	-21.8, 43.4	2			
		Andavadoaka (61 E)	-22.1, 43.3	2 8			
		Ifaty (62 F)	-23.2, 43.6	2			
		Toliara Tsiongobory (63 F)	-23.3, 43.6	2			
		Tsimanampetsotsa (66 G)	-24.0, 43.7	3 0			
		Nosimborona (68 G)	-25.1, 44.1	2			
Madagascar plover <i>Charadrius thoracicus</i> Eberhart-Phillips et al., 2015		Boanamary (51 A)	-15.8, 46.3	2			
		Mahavavy (52 A)	-15.8, 45.8	1 3			
		Marambitsy (53 A)	-15.9, 45.7	1 7			
	N/A	Ankazobe (55 B)	-17.3, 44.1	3	11,100	Monogamous	Resident
		Kirindy Mite (57 C)	-20.9, 43.9	7			
		Mangoky (59 C)	-21.7, 43.4	3			
		Andavadoaka (61 D)	-22.1, 43.3	2 4			
		Ifaty (62 E)	-23.2, 43.6	4			
		Anakao (64 F)	-23.7, 43.7	3			
		Besambay (65 F)	-24.0, 43.7	5			
		Tsimanampetsotsa (66 F)	-24.0, 43.7	2 8			
		Andranomasy (67 F)	-24.2, 43.7	3			
white-fronted plover <i>Charadrius marginatus</i> dos Remedios, 2013 (African mainland)	<i>marginatus</i>	Namibia (49 Z)	-22.6, 14.5	1 8			
	<i>marginatus</i>	South Africa (50 Y)	-34.1, 18.4	1 1		Monogamous	Resident
	<i>tenellus</i>	Marambitsy (53 A)	-15.9, 45.7	3 9	206,300		

Eberhart-Phillips et al., 2015 (Madagascar)	<i>tenellus</i>	Namikia (54 A)	-15.9, 45.8	3			
	<i>tenellus</i>	Kirindy Mite (57 B)	-20.7, 43.9	18			
	<i>tenellus</i>	Fanjakana (58 C)	-21.7, 45.1	3			
	<i>tenellus</i>	Andavadoaka (61 D)	-22.1, 43.3	32			
	<i>tenellus</i>	Tsimanampetsotsa (66 E)	-24.1, 43.8	24			
chestnut banded plover	<i>venustus</i>	Kenya (46 A)	-1.9, 36.3	12			
<i>Charadrius pallidus</i>	<i>venustus</i>	Tanzania (47 A)	-2.9, 35.9	12	301,000	Monogamous	Resident
Eberhart-Phillips et al., 2015	<i>pallidus</i>	Namibia (48 B)	-22.6, 14.5	39			

Table 2. Patterns of isolation-by-distance across ten *Charadrius* plovers. Rousset's linearised F_{ST} was used as genetic distance in Mantel tests. r = Mantel test regression coefficient. Significant isolation-by-distance values ($p < 0.05$) indicated with *.

Plover species	r	F_{ST} gradient
Kentish	0.19	7.15E-07
Kittlitz's	-0.28	-6.71E-05
mountain	0.74	1.37E-05
snowy	-0.10	-3.90E-06
white-fronted	0.40*	4.37E-05
Madagascar	0.16	4.60E-05
piping	0.76*	6.57E-05
common ringed	0.28*	3.86E-06

chestnut-banded	0.99	0.000208
killdeer	0.98	5.42E-05

Figure Legends – high resolution colour images are uploaded in PDF version.

Figure 1. Sampling locations of plover populations for genetic differentiation analyses. Numbers refer to population information (Table 1). In Madagascar insert, symbols do not represent species but rather they show position of sites in North, Middle and South Madagascar.

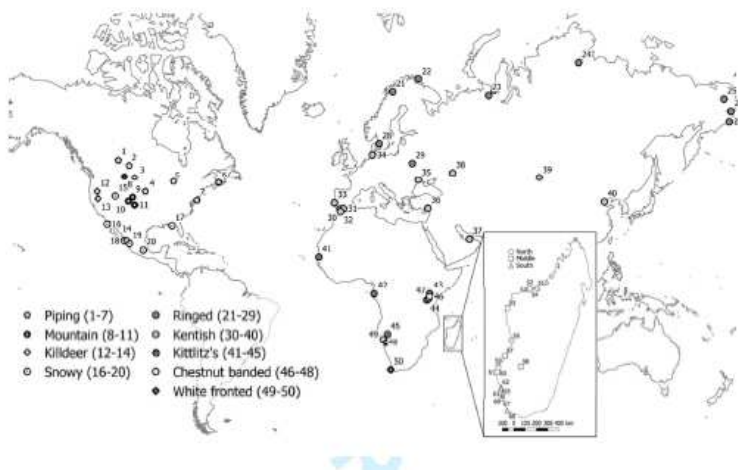


Figure 2. Bayesian population clustering of *Charadrius* plovers according to genetic differentiation in (A) polygamous and (B) monogamous plover species. Migratory species are indicated by asterisk, otherwise a species is an all year resident. Each vertical line represents an individual, colours represent the membership proportion to a given genetic cluster. Models with two or three clusters are presented. See table 1 for site ID number for each species.

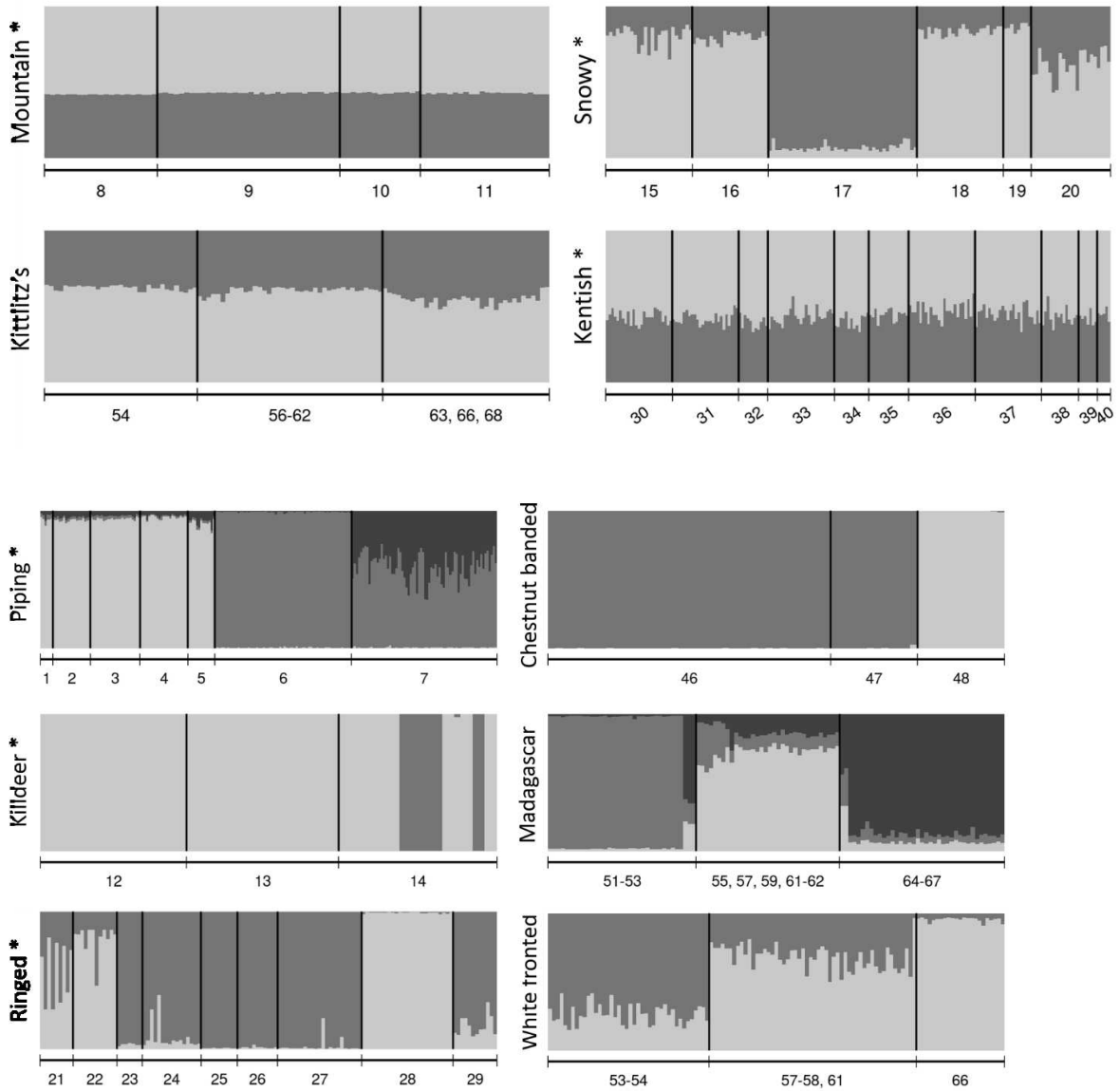


Figure 3. Isolation-by-distance gradient of monogamous ($N = 6$) and polygamous ($N = 4$) *Charadrius* plovers.

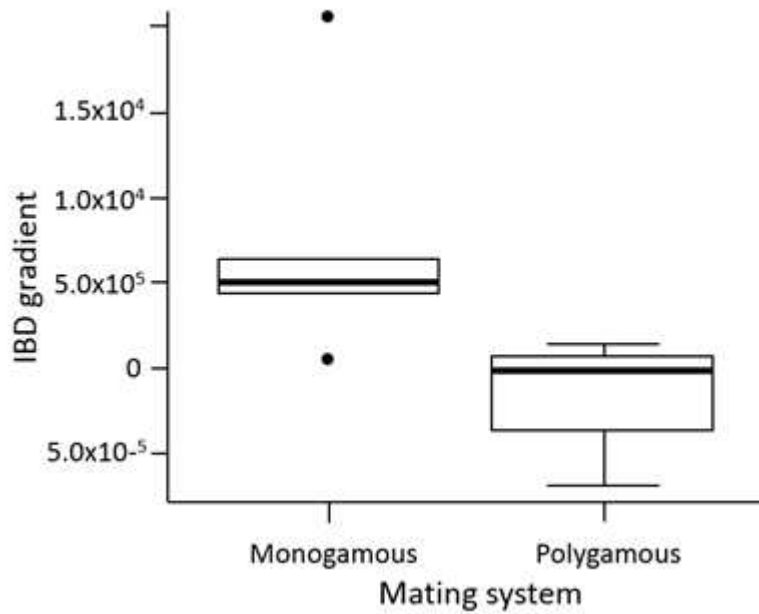


Figure 4. Subspecies richness of monogamous (N = 108) and polygamous (N = 28) shorebird species (Order: *Charadriidae*; suborders: *Charadrii*, *Chionidi*, *Scolopaci* and *Thinocori*).

