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Within-group relatedness is correlated with colony-level social structure and reproductive sharing in a social fish

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Abstract

In group-living species, the degree of relatedness among group members often governs the extent of reproductive sharing, cooperation and conflict within a group. Kinship among group members can be shaped by the presence and location of neighbouring groups, as these provide dispersal or mating opportunities that can dilute kinship among current group members. Here, we assessed how within-group relatedness varies with the density and position of neighbouring social groups in *Neolamprologus pulcher*, a colonial and group-living cichlid fish. We used restriction site-associated DNA sequencing (RADseq) methods to generate thousands of polymorphic SNPs. Relative to microsatellite data, RADseq data provided much tighter confidence intervals around our relatedness estimates. These data allowed us to document novel patterns of relatedness in relation to colony-level social structure. First, the density of neighbouring groups was negatively correlated with relatedness between subordinates and dominant females within a group, but no such patterns were observed between subordinates and dominant males. Second, subordinates at the colony edge were less related to dominant males in their group than subordinates in the colony centre, suggesting a shorter breeding tenure for dominant males at the colony edge. Finally, subordinates who were closely related to their same-sex dominant were more likely to reproduce, supporting some restraint models of reproductive skew. Collectively, these results demonstrate that within-group relatedness is influenced by the broader social context, and variation between groups in the degree of relatedness between dominants and subordinates can be explained by both patterns of reproductive sharing and the nature of the social landscape.

Keywords: cooperative breeder, dispersal, neighbour, *Neolamprologus pulcher*, reproductive skew, restriction site-associated DNA sequencing

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Introduction

Relatedness among group members strongly influences social and reproductive dynamics (Hamilton 1963; Keller & Reeve 1994; Kokko *et al.* 2002). Variation in the average degree of within-group relatedness seems to map onto species-level differences in cooperative tendencies (Cornwallis *et al.* 2010) and accounts for the evolution of maternal allocare across phylogenetically distinct groups of species (Briga *et al.* 2012). Within species, groups with low levels of kinship between group members are expected to have increased within-group aggression (Cant & Johnstone 2000) and increased reproductive sharing among group members

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(Vehrencamp 1983a,b; Keller & Reeve 1994; Whittingham *et al.* 1997; but see Johnstone & Cant 1999). Further, in cooperatively breeding species, low relatedness between dominant breeders and subordinate helpers is usually correlated with reduced allocare by subordinates (Griffin & West 2003; Schneider & Bilde 2008; Nam *et al.* 2010; although see Stiver *et al.* 2005 and Zöttl *et al.* 2013 for exceptions). Consequently, given that kinship among group members drives social and reproductive dynamics within groups, exploring factors that promote variation in within-group relatedness can help us better understand why group dynamics differ both within and across populations.

The social structure beyond the level of the single group, specifically the number and relative location of neighbouring groups, likely contributes to variation in the degree of kinship among group members observed across groups. Within-group relatedness likely decreases with high levels of extra-pair reproduction (Boomsma 2007; Cornwallis *et al.* 2010) and with high turnover in group membership via subordinate dispersal and joining of unrelated immigrants (Dierkes *et al.* 2005). Both group turnover and extra-pair parentage are often dependent on the social organization above the level of the group. For instance, having many close neighbours is often correlated with increased extra-pair parentage (Westneat & Sherman 1997) and is likely correlated with an increased ability of individuals to move between groups, as dispersers often move to adjacent groups (Russell & Rowley 1993; Doolan & Macdonald 1996; Heg *et al.* 2008). Thus, individuals living in areas with high group density may experience lower within-group relatedness relative to groups in less dense areas, where between-group movement and extra-pair mating may be more challenging. In addition to the density of neighbouring groups, the relative location of groups on the edge vs. centre of a colony can influence patterns of within-group relatedness. In many colonial species, territories on the edge of the colony are suboptimal because these groups suffer increased predation, higher rates of mortality of current group members and increased rates of extra-group paternity (Brown & Brown 1987; Forster & Phillips 2009; Hellmann *et al.* 2015a). For all of these reasons, we expect to see lower levels of kinship among group members at the edge of the colony compared to groups in the centre of the colony.

Because there is evidence to suggest that relatedness among group members is not solely driven by forces within the group, we sought to understand how within-group kinship is altered by colony-level social structure in *Neolamprologus pulcher*, a cooperatively breeding cichlid fish native to Lake Tanganyika, East Africa. These fish form territorial groups comprised of a dominant breeding pair and 1–20 subordinates that form size-based dominance hierarchies (Wong & Balshine 2011). Individual social groups are located in colonies of 2–200 groups (Stiver *et al.* 2007). While subordinate females often attain breeding status by inheriting their natal territory, subordinate males typically disperse to fill vacant breeding positions in other territories (Balshine-Earn *et al.* 1998; Stiver *et al.* 2007; Wong & Balshine 2011). Relatedness varies widely between and among groups (Stiver *et al.* 2005), likely because *N. pulcher* social groups have high levels of extra-pair parentage (Hellmann *et al.* 2015a), frequent dispersal between groups (Stiver *et al.* 2007) and high rates of group member turnover (Dierkes *et al.* 2005). These characteristics make this species an ideal system for understanding how colony-level social structure promotes variation in relatedness among group members.

While microsatellite loci have often been used to assess within-population relatedness, techniques that generate genomic-scale data sets, such as restriction site-associated DNA sequencing (RADseq) methods, are increasingly being used to assess genetic variation between populations or species (e.g. Wagner *et al.* 2013; Rasic *et al.* 2014; Viricel *et al.* 2014). By identifying variation in single nucleotide polymorphisms (SNPs) adjacent to restriction enzyme sites, RADseq data yield thousands of polymorphic, homologous SNPs which allow for the rapid acquisition of high-resolution genomic data without requiring any previous information about the genome (Baird *et al.* 2008). These features suggest that this novel technique has great potential for fine-scale analyses of relatedness in behavioural ecology research. Here, we assess this potential by using RADseq data to explore how within-group relatedness varies in relation to the density of nearby social groups and to the location of a group on the colony edge vs. colony centre. We also investigate whether within-group relatedness is linked to patterns of reproductive sharing observed in *N. pulcher* and compared relatedness values generated from RADseq data to those generated from microsatellite data. These analyses will help shed light on the extent to which loci derived from RADseq data might improve the precision of within-population relatedness estimates.

We predicted that within-group relatedness would be lower in denser areas of the colony where subordinates may more easily move between groups (Heg *et al.* 2008; although see Jungwirth *et al.* 2015) and in groups on the edge of the colony where dominant males lose more paternity relative to dominant males in the centre of the colony (Hellmann *et al.* 2015a). These effects on relatedness were expected to be stronger for male than for female *N. pulcher* because vacant positions in the group are more likely to be filled by immigrants for males

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than for females (Stiver *et al.* 2006), breeder turnover is more frequent for males than for females (Stiver *et al.* 2004; Dierkes *et al.* 2005; Jungwirth *et al.* 2016) and extra-pair paternity is more common than extra-pair maternity (Hellmann *et al.* 2015a). Finally, we predicted that rates of extra-pair parentage would be higher when the dominant male and female breeding pair were more related in order to reduce potential costs associated with inbreeding depression (Arct *et al.* 2015). However, we did not predict to see a similar relationship with subordinate reproduction, because subordinate reproduction is more highly constrained and a laboratory study in this species found that the degree of subordinate reproduction did not vary with relatedness to dominants (Bruintjes *et al.* 2011).

Methods

Study site and field collection

From February to April 2013, we collected tissue samples from wild *Neolamprologus pulcher* groups in Kasakalawe Bay, Lake Tanganyika, East Africa (8°46′S; 31°46′E), using SCUBA. Groups were dispersed among seven colonies at depths ranging from 11 to 13.5 m. Each colony consisted of many (7 to ~200) distinct social groups each defending a discrete territory. Colonies were separated from each other by large open expanses of sand and rubble uninhabited by *N. pulcher* (Stiver *et al.* 2007). Each focal group was observed prior to sampling to identify dominant and subordinate group members. An individual was considered to belong to the focal group if it swam repeatedly under the rocks within the group's territory without eliciting aggression from other fish in the territory. Within each focal group, dominant and subordinate *N. pulcher* were differentiated by size, as dominance is very strongly linked to body size in this species (Reddon *et al.* 2011; Dey *et al.* 2013) and the largest male and female *N. pulcher* are almost always the dominant pair (Wong & Balshine 2011). Parentage analysis of fry from these groups confirmed that the dominant individuals were correctly identified and collected in conjunction with their home territory (Hellmann *et al.* 2015a). All conspecific neighbours within a 3 m radius were mapped in relation to the sampled focal group. Groups were defined as being on the edge of the colony if unoccupied areas bordered half or more of the group's territory (i.e. there were no conspecific groups within 10 m on that side of the territory; Hellmann *et al.* 2015a). For each focal social group, all sexually mature individuals, as well as those close to sexual maturity (>30 mm standard length (SL): the length from the tip of the snout to the base of the caudal fin; Taborsky 1985), were captured using fence nets and hand nets. Dominants and subordinates that had been captured were then taken to the surface in mesh bags where they were weighed to the nearest 0.001 g and measured to the nearest 0.01 mm SL. Fish were euthanized by immersion in a lethal concentration of benzocaine (ethyl p-aminobenzoate, 1.0 μg/mL) for use in other studies (Hellmann *et al.* 2016). Sections of the dorsal fin were also taken from all individuals to be used as a genetic sample for relatedness analysis for this study.

RAD methods

A total of 31 groups (20 centre groups, 11 edge groups) with 171 individuals (31 dominant females, 30 dominant males, 51 subordinate females, 44 subordinate males and 15 reproductively immature subordinates) were analysed (see Table S1, Supporting information for distribution of dominants and subordinates across colonies). While we collected more than 31 groups (Hellmann *et al.* 2015a, 2016), we limited the analyses to these 31 groups because (i) we were confident that group members were correctly collected from these groups, (ii) they contained two or more reproductively mature subordinates and (iii) samples from these groups produced sufficiently high-quality DNA for generating RADseq data. Individually barcoded RAD libraries were generated using the protocol of Sovic *et al.* (2016) with the following modifications. *Eco*RI and *Pst*I high-fidelity restriction enzymes (New England Biolabs) were used for digestion, and 250 ng of genomic DNA was digested for 90 min at 37 °C and then heat-inactivated for 20 min at 80 °C. Samples were quantified with qPCR prior to library amplification, and a minimum threshold of 1×10^6 molecules was required for each sample to help reduce possible effects of low library complexity. Samples not meeting this threshold were re-prepped prior to sequencing, as preliminary data suggested that samples below this value showed relatively high levels of missing data. Samples were sequenced in pooled libraries of up to 36 individuals and sequenced in single-end 50-bp runs on an Illumina HiSeq 2500.

Bioinformatics methods

Demultiplexing, quality filtering, locus assembly and genotyping were performed with AftrRAD v4.1 (Sovic *et al.* 2015) using default parameters, with the exception that the 'reargument' was set to 'TGCAG' to match the use of the restriction enzyme *Pst*I. The default parameters include a 90% mismatch allowance between alleles when assembling loci, a minimum read depth of 10 for each allele for genotyping and a minimum Phred score of 20 for each base in order for reads to be retained for

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analysis. Levels of missing data were assessed for each sample, and three individuals were removed from the data set due to relatively high levels of missing data that appeared to be associated with low sequencing coverage/depth. Reads were screened for the build-up of artifactual SNPs at the end of reads; any SNPs beyond position 35 (after removing barcode and restriction sites at the beginning of reads) were omitted in this data set. Paralogous loci were identified based on excess heterozygosity and the presence of more than two alleles in an individual at a given locus. In total, we identified and removed 2453 paralogous loci from the data set. We also identified 40 447 monomorphic loci, which were not analysed further, and 14 101 polymorphic loci. Of the polymorphic loci, 2250 loci were scored in 100% of the 168 individuals remaining in our data set. These 2250 loci were then used to assess relatedness.

Statistical analysis

Relatedness was assessed using the package RELATED (Pew *et al.* 2015), which implements the code for COANCESTRY (Wang 2002) in R. We used the measure of relatedness described by Wang (2002) because it better accounts for biases often associated with small sample sizes and samples that include clusters of relatives (i.e. groups of parents and offspring), which are characteristics of this data set (Konovalov & Heg 2008). Further, when allele frequencies are calculated relative to subpopulations (rather than the population as a whole), the relatedness values produced by Wang (2002) are more accurate (minimize root-mean standard error) than the measures of relatedness described by Queller & Goodnight (1989), Milligan (2003) and Wang (2011). To account for population structure, the data set was split into four subpopulations, or groupings of colonies located in close proximity to each other (see Fig. 1), and relatedness among group members was analysed relative to the subpopulation (Wang 2011). However, the reference population had little influence on relatedness values. All estimates of relatedness between the dominant female and subordinates and 107/109 estimates of relatedness between the dominant male and subordinates were binned into the same category (R = 0, 0.125, 0.25, or 0.5; see below) when using the whole population as the reference population compared to using the subpopulation as the reference population.

We used cumulative link mixed models (CLMM) to test predictors of a given subordinate's relatedness to the dominant male and female in its group (R package 'ordinal'; Christensen 2012). For these models, all relatedness values were categorized as 0 (or below 0), 0.12,

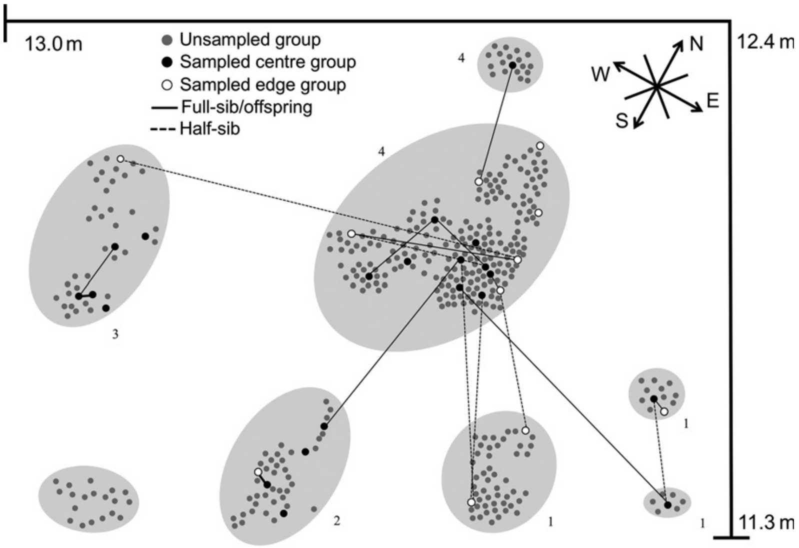

Fig. 1 Partial map of the seven sampled colonies (and one additional unsampled colony), with unsampled groups represented as grey dots and sampled groups as black (centre groups) and white (edge groups) dots. After removing three individuals from our data set due to low sequencing coverage, we analysed a total of 22 dominants and 37 subordinates across 11 edge groups and 37 dominants and 72 subordinates across 20 centre groups. Lines connect kin found in different groups within the same colony, as well as different groups in different colonies. Solid lines indicate full-siblings/offspring between two groups, and dashed lines indicate half-siblings between two groups. Depths of the colonies are identified on the bars lining the colony map. Numbers next to the colonies indicate groupings of the colonies into four subpopulations for analysis. Note that distances between the colonies on the figure are not to scale, and there are additional unsampled groups that are not depicted on the map.

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0.25 and 0.5. We analysed relatedness values as discrete values rather than continuous values for two reasons. First, while we know that *N. pulcher* can discriminate between relatives and nonkin (Le Vin *et al.* 2010), we do not know whether individuals in this species can discriminate between unrelated individuals that share greater or fewer genes relative to the population average. Therefore, we felt that binning values into discrete categories, especially binning *R*-values between −0.5 and 0 into one category, may be more biologically relevant given what we know about this species and given the hypotheses we sought to address. Second, point estimates of relatedness for dominant males and subordinates were not normally distributed, and a normal distribution could not be achieved through data transformation. However, as models examining continuous relatedness values between dominant females and subordinates fit well, we include those results in the Supporting information to demonstrate that at least for those data, the same patterns emerge when analysing either continuous or binned data.

Values were categorized by evaluating 95% confidence intervals around the point estimates of relatedness and placing data points into the category (0, 0.12, 0.25 or 0.5) that was within the confidence interval. For all data points, confidence intervals were small enough that they did not span more than one category. In one case, the calculated confidence interval did not encompass any of the categories (was between 0.25 and 0.5, but not inclusive of either value), and so, we conservatively assigned this data point to the 0.25 category. In the models, we tested independent variables describing the spatial location of the group (categorical: centre or edge of the colony), the density of neighbouring groups (continuous: the number of neighbouring groups within a 3 m radius), subordinate sex and relative size of the dominant and subordinate [(dominant SL − subordinate SL)/dominant SL]. The focal group of the subordinate (nested within colony) and the colony of the focal group were both included in the model as random effects. We chose to test the number of neighbouring groups within a 3 m radius of the focal group because subordinate *N. pulcher* preferentially visit neighbouring groups within a 3-m radius of their own group (Heg *et al.* 2008). The density of neighbouring groups was not correlated with a group's location on the edge vs. centre of the colony (general linear model: $T_{29} = -0.61$, $P = 0.55$), as many colonies have hard edges with dense areas that end abruptly whereas other have sparser areas that gradually thin out.

Because we also had information on extra-pair parentage for the majority of these groups (28/31 groups; see Hellmann *et al.* 2015a), we used generalized linear mixed models (GzLMM) with a binomial distribution to determine (i) whether a subordinate's likelihood of reproducing within its group was predicted by its relatedness to the dominants in its group and (ii) whether the relatedness between the dominant male and female pair predicted the level of extra-pair parentage in the group.

Finally, to examine general dispersal patterns, we examined the relatedness of male and female subordinates to subordinates within their own subpopulation (Fig. 1), as well as to subordinates in the other subpopulations. Because dispersal tends to be size biased (Stiver *et al.* 2007), we split subordinates into two size classes according to those suggested by Stiver *et al.* (2007): small reproductively mature subordinates (30–45 mm SL) and large reproductively mature subordinates (45–55 mm SL).

Comparisons of RADseq and microsatellite data sets

For 107 individuals in this data set, we could quantify relatedness using both RADseq data (described above) and six highly variable microsatellites used in previous analyses of relatedness in this fish (Hellmann *et al.* 2015a; see Table 1). We assembled two data sets, each using the same 107 individuals, one with relatedness data generated from using six microsatellite loci and the other with the same 2250 RADseq loci used in the full data set. We ran the previously described relatedness analysis on both data sets (package RELATED using the Wang (2002) measure of relatedness), and compared both the reported point estimates and confidence intervals of relatedness values. All loci in both data sets were within Hardy–Weinberg equilibrium.

Ethical note

Neolamprologus pulcher is a highly abundant cichlid species and is neither endangered nor threatened; however, we made attempts to reduce the number of fish we collected by using fish for multiple studies that addressed different questions (Hellmann *et al.* 2015a, 2016). New social groups had occupied the vacated territories within a day or two postcollection. All methods, including euthanasia techniques, were approved by The Ohio State University IACUC (protocol ID 2008A0095) and the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol Number 10-11-71). Our procedures adhered to the guidelines of the Canadian Council for Animal Care and the Animal Behavior Society.

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Table 1 Genetic characteristics of the six loci used to run the relatedness analysis based on microsatellites. Shown are observed (H_{obs}) and expected (H_{exp}) heterozygosity, the polymorphic information contents (PIC) and the estimated frequency of null alleles for each locus. Heterozygosity, PIC and null frequencies were calculated using CERVUS 3.0 based on genetic data from the 54 dominants from the reduced data set

Locus	References	Alleles	Range	H_{obs}	H_{exp}	PIC	Null
LOC101	Brandtmann *et al.* (1999)	21	150–195	0.720	0.875	0.853	+0.0916
TMO11	Zardoya *et al.* (1996)	24	170–230	0.889	0.885	0.869	−0.0079
TMO13	Zardoya *et al.* (1996)	21	220–280	0.850	0.855	0.843	+0.0184
TMO25	Zardoya *et al.* (1996)	18	360–415	0.815	0.832	0.808	+0.0041
UME003	Parker & Kornfield (1996)	28	190–265	0.944	0.913	0.900	−0.0229
US783	Schliewen *et al.* (2001)	25	160–250	0.852	0.924	0.910	+0.0327

Results

Within-group relatedness and colony structure

Consistent with previous studies in this species (Dierkes *et al.* 2005; Stiver *et al.* 2005), we found that subordinates were more closely related to the dominant female in their group (mean ± SE: 0.16 ± 0.03) than to the dominant male in their group (mean ± SE: 0.02 ± 0.02; Wilcoxon rank sum: $W = 3535$, $P < 0.001$). Smaller subordinates were more closely related to the dominant female than were larger subordinates (CLMM: $Z = 3.79$, $P < 0.001$; Fig. 2), but the body size of a subordinate did not influence its relatedness to its dominant male (CLMM: $Z = 0.34$, $P = 0.73$). Interestingly, subordinate males were more related to the dominant female than were subordinate females (male subs 0.19 mean ± 0.04 SE, female subs 0.05 mean ± 0.04 SE; $Z = 2.07$, $P = 0.04$) and tended to also be more related to dominant males than were subordinate females (male subs 0.03 mean ± 0.03 SE, female subs −0.02 mean ± 0.02 SE; $Z = 1.62$, $P = 0.11$).

Subordinates (both males and females) were more closely related to their dominant male breeders in groups located in the centre of the colonies compared to groups along the edges of the colonies (Wilcoxon rank sum: $W = 823$, $P = 0.01$; Fig. 3). In general, we observed extremely low levels of relatedness between dominant males and subordinates for groups on the edges of the colonies: 35 of the 37 subordinates collected from edge territories were completely unrelated to the dominant male in their group (mean relatedness values ranging from −0.13 to 0.01). Relatedness between dominant females and subordinates did not vary between the centre and edges of the colonies (CLMM: $Z = 0.76$, $P = 0.45$). Relatedness between dominant females and their subordinates was negatively correlated with the density of neighbouring groups ($Z = -2.08$, $P = 0.04$; Fig. 4), while relatedness between dominant males and subordinates was not related to the density of neighbouring groups ($Z = 1.06$, $P = 0.29$).

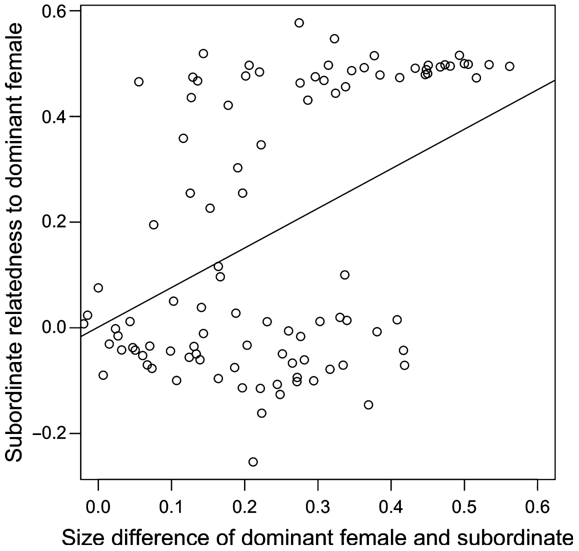

Fig. 2 The size difference between the dominant female and the subordinate (larger values = larger size difference and smaller subordinates; size difference of zero means that dominant females and subordinates were the same size) plotted against the relatedness values of subordinates to the dominant female within their group. The graph demonstrates that subordinates were significantly less related to dominant females within their group when there was a small size difference between the dominant female and subordinate.

Within-group relatedness and extra-pair parentage

Extra-pair paternity, by subordinate males from the same group or by dominant and subordinate males from neighbouring groups, was significantly less likely when the relatedness between the dominant male and female was high (GzLMM with binomial distribution: $Z_{16} = -3.20$, $P = 0.001$). The frequency of extra-pair maternity was not correlated to the degree of relatedness between the dominant male and female ($Z_{16} = 0.003$, $P = 0.99$). Subordinate males were significantly more likely to reproduce in their group when

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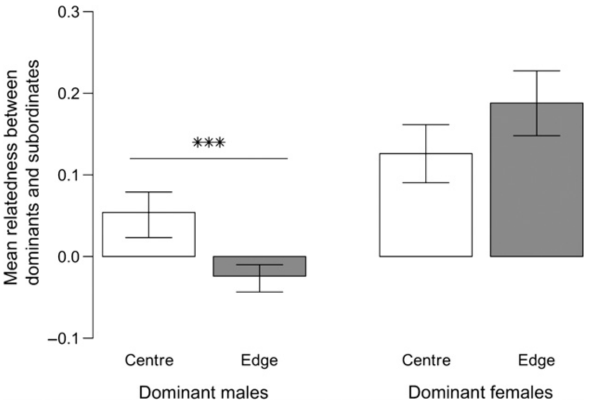

Fig. 3 Mean relatedness (±standard error) between dominants and subordinates for groups in the centre of the colony vs. on the edge of the colony. This figures shows that subordinates were significantly more related to the dominant breeding male in their group when groups were located in the centre of the colony compared to the edge of the colony, but relatedness between dominant females and subordinates did not vary significantly between groups on the centre and edge of the colony.

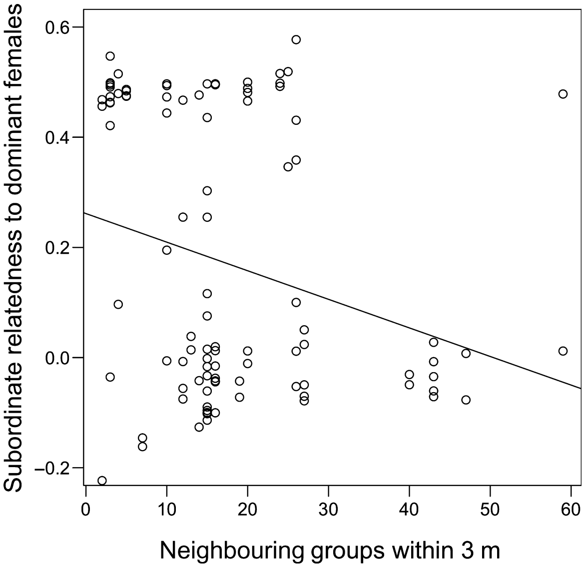

Fig. 4 The number of neighbouring social groups within a 3 m radius plotted against the relatedness values of subordinates to the dominant female within their group. The graph demonstrates that as the density of neighbouring groups increased, subordinates were significantly less related to dominant females within their group.

they were more related to the dominant male (GzLMM with binomial distribution: $Z_{14} = 2.00$, $P = 0.04$), and subordinate females were significantly more likely to reproduce if they were more closely related to their dominant female ($Z_{18} = 2.72$, $P = 0.006$).

Movement between groups and colonies

Restriction site-associated DNA sequencing allowed us to track individual movement among groups and colonies (Fig. 1). Smaller female subordinates were more likely to be related to other members of their current group than were larger female subordinates (GzLMM with binomial distribution: $Z_{21} = 1.96$, $P = 0.05$). Body size of male subordinates did not influence the probability of being related to current group members ($Z_{17} = 0.58$, $P = 0.56$).

Nearly 5% (8/168) of the individuals in our data set had relatives in a different group within the same colony and another 5% (8/168) had kin in a group within a different colony altogether. In general, small female and male subordinates (30–45 mm SL) were more related to other small female and male subordinates (respectively) within their own subpopulation than to small subordinates in other subpopulations (Wilcoxon rank sum; small females: $W = 14\ 766$, $P < 0.001$; small males: $W = 12\ 396$, $P = 0.002$). There was a similar, albeit weaker and nonsignificant, trend for large female subordinates to also be more related to large female subordinates in their own subpopulation (45–55 mm SL: $W = 3400$, $P = 0.08$). However, large male subordinates were no more related to large male subordinates within their subpopulation compared to large male subordinates in other subpopulations ($W = 290$, $P = 0.58$).

Comparison of RADseq and microsatellite data for estimating relatedness

Restriction site-associated DNA sequencing was a much more precise technique for measuring and tracking relatedness compared to microsatellite markers. While point estimates of relatedness values were correlated between the two techniques (Pearson's correlation: $t_{2344} = 10.9$, $P < 0.001$), the correlation coefficient was relatively low (Pearson's $r = 0.22$). Confidence intervals were also significantly larger with the microsatellite data than with RADseq data (Wilcox rank sum: $W = 479\ 300$, $P < 0.001$; Fig. 5). In some cases, confidence intervals were nonoverlapping between the two techniques and microsatellites provided different estimates of relatedness than RADseq. Our ability to distinguish unrelated individuals from related individuals was reduced when using the microsatellite data (Fig. 5). For example, of the 53 subordinates that were present in both data sets, RADseq data classified 35 as unrelated to the dominant female, six as a half-sibling of the dominant female and 12 as a full-sibling or offspring of the dominant female. Confidence intervals in all cases were small enough that classifications into these categories were not ambiguous (i.e. confidence intervals

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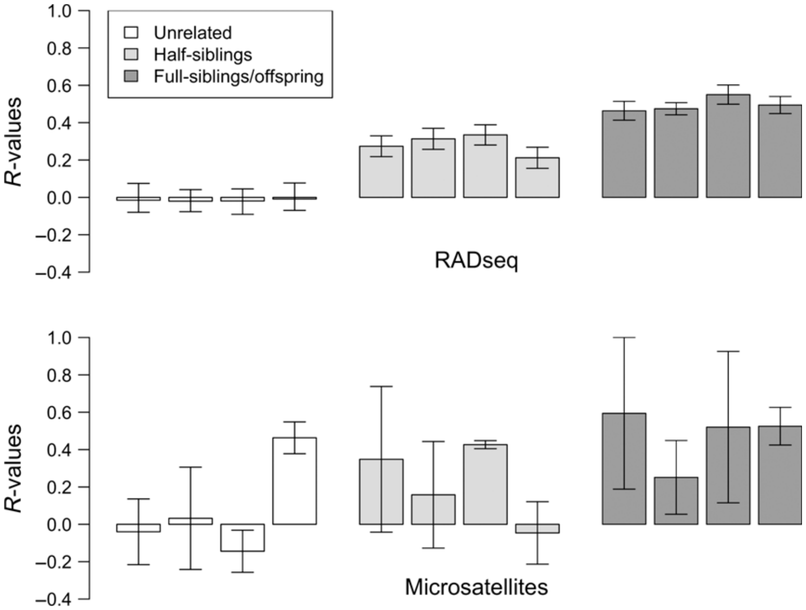

Fig. 5 Using restriction site-associated DNA sequencing (RADseq), we identified unrelated individuals from different groups (white), half-sibs from different groups (light grey) and full-sibs/offspring from different groups (dark grey). Data presented are means with 95% confidence intervals. RADseq techniques significantly improve the precision of relatedness estimates compared to microsatellites, which provided much larger confidence intervals.

did not span multiple categories). However, using microsatellite loci, only 22 of 53 subordinates had confidence intervals small enough that subordinates could unambiguously be assigned as unrelated, half-siblings or full-siblings/offspring of the dominant female. For 20 subordinates, confidence intervals when using microsatellites were large enough that we were unable to distinguish between subordinates that were unrelated to the dominant female vs. those that had a half-sibling relationship with the dominant female. For an additional four subordinates, we were unable to distinguish between subordinates that had a half-sibling relationship with the dominant female vs. those that were full-siblings/offspring of the dominant female. For the remaining seven subordinates, microsatellites classified the relationship between dominant females and subordinates differently than did the RADseq data.

Discussion

Using RADseq data, we generated over 2000 polymorphic loci and used these to detect novel patterns of relatedness in *Neolamprologus pulcher*, an emerging model species for the study of cooperation and social behaviour. First, within-group relatedness varied with both the density of neighbouring groups and the location of the focal group on the edge vs. centre of the colony, demonstrating that colony-level social structure can help explain variation in the degree of relatedness of group members. Further, we found that subordinates were more likely to reproduce when they were related to their same-sex dominant, corroborating restraint models of reproductive skew (Johnstone & Cant 1999). Finally, we found evidence that subordinate females disperse frequently, as they were less related to dominants in their group than subordinate males. However, given that subordinate females tended to be more related to subordinates within their subpopulation compared to subordinates in other subpopulations, female subordinates likely disperse relatively short distances.

Relatedness and colony-level social structure

We found that both the density of neighbouring groups and the location of a group on the edge vs. centre of the colony were connected to the degree of relatedness between dominants and subordinates in a group. Dominant males were significantly less related to subordinates within their group when their group was on the edge of the colony compared to when groups were in the centre of the colony; however, relatedness between dominant females and their subordinates did not vary between territories on the edge and in the centre of the colony. This colony-level variation in relatedness between dominant males and subordinates, but not dominant females and subordinates, suggests that it is the movement and behaviour of the dominant male that is driving these differences between the centre and edge of the colony, rather than the behaviour of the subordinates. We suggest two nonmutually exclusive reasons why we might see these patterns. First, rates of extra-group maternity do not vary between the edge and centre of the colony, but rates of extra-group paternity are higher on the edge of the colony than the centre of the colony (Hellmann *et al.* 2015a), thereby reducing the number of offspring that are descendants of the dominant male. However, this alone does not likely explain the extremely low levels of relatedness on the edge of the colony, given that dominant males on the colony edge still sire most of the offspring on their territory (Hellmann *et al.* 2015a). Second, it is likely that increased predation on the colony edge on dominants is male biased. Males engage in extensive fights over territory ownership (O'Connor *et al.* 2015), which can make dominant males more vulnerable to predation and can reduce their tenure as a dominant. Therefore, these results suggest that males on the edge of the colony face particularly high fitness costs of living on the edge of the colony, whereas females do not appear to share these same fitness consequences of living on the colony edge.

Consistent with our predictions, we found that subordinates were less related to dominant females within their group when their group was located in a denser area of the colony. However, we did not find any effect of density on relatedness to the dominant male. These contrasting patterns may be due to differences in the ways in which males and females acquire and hold territories in this species. In this species, females hold only one territory, whereas males often hold multiple territories, particularly when territories are spatially clustered together (i.e. in denser areas; Desjardins *et al.* 2008; Wong *et al.* 2012). Therefore, in denser areas, it is possible that subordinates may be able to more easily disperse from their natal territory and move to another territory of their father. This may be advantageous, as subordinates may face less aggression joining a group of their father compared to a group containing no kin (Watson *et al.* 1994). In these situations, subordinates would be related to the dominant male in the group, but would be unrelated to the dominant female in the group. Subordinate males in a closely related species (*Neolamprologus obscurus*) seem to adopt this strategy: they disperse from the territory of their mother, but remain within the larger territory of their father (Tanaka *et al.* 2015).

Relatedness and reproductive sharing

We found evidence that patterns of reproductive sharing among groups are related to variation in within-group relatedness in this species. First, extra-pair paternity was significantly less likely when the dominant male and female were more related. This is in contrast to our predictions and to the results of a meta-analysis recently conducted on birds (Arct *et al.* 2015); however, given that no dominant pair had relatedness beyond the level of cousins, it is likely that higher relatedness between dominants in our data set did not produce inbreeding depression, but did provide benefits in terms of increasing the number of their complementary gene complexes genes passed to their offspring (optimal outbreeding distance: Bateson 1982; Kokko & Ots 2006). It is also possible that other nongenetic benefits of breeding with kin (e.g. increased cooperation during parental care) outweigh any potential costs or risk associated with inbreeding depression (Thünken *et al.* 2007).

Both subordinate males and subordinate females were more likely to reproduce when they were more related to their same-sex dominant. These results are in contrast to concession and tug-of-war models of reproductive skew (Vehrencamp 1983b; Keller & Reeve 1994; Hamilton 2013), but corroborate restraint models of reproductive skew (Johnstone & Cant 1999). In restraint models, the degree of reproductive skew reflects the credibility of dominant threats of eviction. Because dominants face higher costs of evicting related subordinates, restraint models predict that related subordinates are better able to successfully reproduce within their group without triggering eviction (Johnstone & Cant 1999). Further, given the frequent level of extra-group parentage in this species (Hellmann *et al.* 2015a), it may be impossible for dominants to completely prevent extra-pair fertilizations due to the high number of reproductive competitors both within their own group and in nearby groups. In these cases, it would benefit the dominant to allow kin to reproduce rather than unrelated group members or neighbours, because dominants gain indirect fitness benefits if the offspring of their kin survive and mate.

Dispersal patterns inferred from relatedness

Despite a widely held notion that subordinate females disperse less frequently than males in these fish (Stiver *et al.* 2006), we found that subordinate females were less related to dominants within their group than were subordinate males. Further, we found that smaller subordinate females were more likely to be related to current group members than larger female subordinates, whereas small and large subordinate males were

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similarly related to current group members. Combined, these results suggest that subordinate females disperse more frequently and at smaller sizes relative to subordinate males. Given that female subordinates are more related to subordinates within their own subpopulation, it is likely that female subordinates generally disperse to nearby groups within their subpopulation, while male subordinates disperse between subpopulations. This is consistent with previous studies in this species that have found evidence for male-biased dispersal between colonies, but no evidence for sex-biased dispersal within a colony (Stiver *et al.* 2007). Anecdotally, in our data set, females accounted for nearly all recorded incidences of movement between groups within a colony, whereas males conducted nearly all recorded incidences of movement between colonies.

We suggest three potential explanations for why subordinate males and females may disperse at different sizes. First, because subordinate females disperse for shorter distances within the colony, it may be easier for them to disperse at a smaller size compared to subordinate males, who often disperse between colonies and must cross large areas of open sand without shelter where they are highly vulnerable to predators (Stiver *et al.* 2007). Second, it is possible that male and female subordinates disperse at the same age, but because males grow more quickly than females (A. Jungwirth, personal communication), male subordinates are larger in body size at the time of dispersal. Finally, it may not be advantageous for small subordinate females to remain in their natal group if there are several larger subordinate females in their group. Because dominant female tenure tends to be relatively long (Stiver *et al.* 2004; Dierkes *et al.* 2005) and dominance is strictly size based, only the largest subordinate female can inherit the territory and any smaller females in the group must wait until she dies before they can become dominant. Therefore, smaller female subordinates in large groups may benefit from establishing themselves in a nearby group with fewer female subordinates, where they have a greater chance of inheriting the dominant position. This may explain why subordinate female dispersal appears to be common despite female inheritance of territories (Stiver *et al.* 2006): the females with the best chance of inheriting their natal territory (large females) do not disperse, whereas females with a low likelihood of inheriting their natal territory disperse to groups where they are more likely to inherit a breeding position. However, because subordinate males rarely inherit territories (Stiver *et al.* 2006), they may wait to disperse from their father's territory until they reach a size at which they may be able to challenge for a dominant breeding position in another group or can opportunistically disperse to a vacant territory (Tanaka *et al.* 2015). Subordinates in the closely related *N. obscurus* appear to adopt similar strategies—subordinate females disperse away from their father's territory at a smaller size, but subordinate males remain in their father's territory, likely because subordinate males are more tolerated in the territory of their father than a territory of an unrelated male (Tanaka *et al.* 2015).

Conclusions

This study is one of the first to use RADseq data to assess within-population relatedness in social species in the wild. Relative to microsatellites, these genomic-scale data provided significantly more precise measurements of relatedness and thus show great potential for studies that estimate relatedness using genetic data in behavioural ecology. Using the thousands of loci generated with this genomic technique, we were able to uncover novel patterns of relatedness in *Neolamprologus pulcher*. Specifically, we found that differences in the degree of relatedness among group members can help explain differences in the level of reproductive sharing between dominant and subordinate group members. Further, we demonstrate that the variation in the density of neighbouring groups, as well as the location of a group on the edge vs. centre of the colony, is linked to patterns of relatedness within a group. These data are in agreement with a growing literature demonstrating that the broader social context is an important determinant of social and reproductive dynamics within a group (Bergmüller *et al.* 2005a,b; Radford 2008; Hellmann & Hamilton 2014; Hellmann *et al.* 2015a,b; Jungwirth & Taborsky 2015). Specifically, neighbouring groups can influence both direct and indirect fitness gains within an individual's own group (Hellmann *et al.* 2015a; Jungwirth & Taborsky 2015) as well as an individual's ability to negotiate additional current and future fitness opportunities (Buston & Zink 2009; Shen & Reeve 2010). Therefore, further research exploring how within and between group dynamics interact would improve our understanding of individual decision-making and reproductive success in group-living species.

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J.K.H. collected field data, conducted the relatedness and statistical analysis and wrote the entire first draft of this manuscript. I.Y.L., C.M.O., A.R.R., S.B., S.E.M. and I.M.H. aided with coordination of the field season and data collection. M.G.S. and H.L.G. aided with relatedness analysis. M.G.S. designed and ran the custom bioinformatics pipeline. All authors aided with manuscript revisions and study design.

Data accessibility

All group and individual information, AftrRAD output and raw read files are available on Dryad: doi: 10.5061/dryad.5632c.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 The distribution of dominant males, dominant females, and subordinates across all edge and center groups in each of the seven sampled colonies.

Appendix S1 The results of general linear mixed models analysing relatedness between dominant females and subordinates using continuous, rather than binned, relatedness values.

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