

Dispersal patterns and status change in a co-operatively breeding cichlid *Neolamprologus pulcher*: evidence from microsatellite analyses and behavioural observations

K. A. STIVER*†, P. DIERKES‡, M. TABORSKY‡§
AND S. BALSHINE*

*Department of Psychology, McMaster University, Hamilton, Ontario, L8S 4K1, Canada, ‡Konrad Lorenz-Institut für Vergleichende Verhaltensforschung (KLIVV), A-1160 Vienna, Austria and §Department of Behavioural Ecology, Institute of Zoology, University of Bern, CH-3032 Hinterkappelen/Bern, Switzerland

(Received 11 July 2003, Accepted 18 March 2004)

Genetic techniques and long-term behavioural observations were combined to investigate dispersal patterns and changes in social position in *Neolamprologus pulcher*, a co-operatively breeding cichlid from Lake Tanganyika. Comparisons of genetic variance (F_{ST}) across sub-populations demonstrated that fish were genetically more similar to individuals from proximate sub-populations compared to individuals from distant sub-populations. Microsatellite analyses revealed year-long philopatry for some individuals and that other individuals dispersed to new territories and sub-populations. Individuals appeared to disperse farther (across many territories in a sub-population or to new sub-populations) to achieve breeding status. Non-breeding group members (or helpers) were observed to inherit breeding positions and male breeders were replaced faster than female breeders. These results demonstrate that important and difficult to obtain life-history information can be obtained from genetic sampling. © 2004 The Fisheries Society of the British Isles

Key words: Cichlidae; genetics; Lake Tanganyika; Lamprologines; social dynamics.

INTRODUCTION

Virtually all living organisms disperse. Patterns of dispersal differ between species, and several models have been proposed to explain how animals migrate. For example, Wright (1931, 1943) proposed a simplified model of dispersal, referred to as the infinite island model (Wright, 1943; Taylor *et al.*, 2000), in which animals randomly disperse to any territory in a population and a more constrained model, in which animals are more likely to disperse to near-by territories, resulting in genetic isolation by distance (Wright, 1943). Another model, known as the stepping-stone model, expanded upon the second Wright model, emphasizing the importance of dispersal distance and included the

†Author to whom correspondence should be addressed. Tel.: +1 905 525 9140 (ext: 26042); fax: +1 905 529 6225; email: stiverka@mcmaster.ca

assumption that individuals can only disperse to neighbouring sites (Kimura, 1994; Irwin & Taylor, 2000).

To explain why animals disperse, cost and benefit analyses are commonly employed. Benefits of dispersal include a potential increase in available resources, avoidance of inbreeding and a reduction in competition between kin (Pusey, 1987). Dispersal, however, is usually risky and individuals need to balance the risks of moving to a new location against these benefits. Typically, one sex disperses further than the other. For example, in most mammals, there is male biased dispersal (Greenwood, 1980). It has been argued that this results from the fact that females (usually the primary if not the sole 'care-givers' in mammals) benefit most from philopatry as familiarity with food sources and shelter can strongly enhance female reproductive success (Pusey, 1987). In addition, since male mammals typically do not care, high quality males can sequester many females, leading to unequal reproductive success among males (Andersson, 1994). Under such conditions, it may pay a male to disperse in an attempt to gain new mates (Pusey, 1987). In contrast, birds, which are typically biparental, generally have a female biased pattern of dispersal. This female biased dispersal has been attributed to the strong influence of territory quality on male reproductive success in birds. Males may benefit most from philopatry as the quality of a male's territory can influence both mate attraction and the survival of young (Pusey, 1987).

This study examined how and why dispersal occurs in *Neolamprologus pulcher* (Trewavas & Poll), a co-operatively breeding cichlid from Lake Tanganyika. These fish live in social groups consisting of at least one dominant breeder of each sex and one to 14 helpers of both sexes (Balshine *et al.*, 2001). Helpers assist breeders by defending the territory from predators and space competitors and maintaining the territory and the brood chamber by digging and removing debris. They also perform direct brood care by fanning the eggs, preventing fungal build up on eggs, and by defending the larvae and free-swimming young (Taborsky & Limberger, 1981; Taborsky & Grantner, 1998; Balshine *et al.*, 2001). *Neolamprologus pulcher* mainly feed on zooplankton in the water column, and do not compete over food. Occasionally, group independent individuals are observed, which are tolerated in particular territories but do not help (S. Balshine & M. Taborsky, pers. obs.).

At the study population at Kasakalawe Bay, each *N. pulcher* group defends a territory (median size 3150 cm²) consisting of clusters of rocks (under which the fish shelter) on a sandy substratum (Balshine *et al.*, 2001). Sub-populations are made up of clusters of adjoining territories inhabited by groups of fish. The sub-populations consist of two to 200 territories (Balshine *et al.*, 2001; M. Taborsky, pers. obs.) and are separated by an average of 22 ± 3 m of uninhabited sand and rock.

In most co-operative breeding species, natal philopatry is common (Hatchwell & Komdeur, 2000): in *N. pulcher*, it has been generally assumed that helpers are relatives of the breeders (Taborsky, 1984, 1985) and that little dispersal occurs (Balshine *et al.*, 2001) but no conclusive data to support this hypothesis have been collected previously. It was expected that if fish dispersed, they would move in a stepping-stone fashion to nearby territories or sub-populations to minimize predation risk.

Poor knowledge of dispersal patterns in *N. pulcher* has made it difficult to compare these co-operatively breeding fish to other extensively studied

co-operative breeding species, such as the Florida scrub jay and the dwarf mongoose, for which dispersal information is known (Woolfenden & Fitzpatrick, 1990; Creel, 1994). It was expected that individual *N. pulcher* would disperse in order to obtain an immediate breeding position or to join a new group in a more dominant position than that previously enjoyed.

As a result of *N. pulcher*'s small size [maximum body mass (wet mass of wild caught fish) = 6.7 g; maximum body standard length (L_S) = 6.6 cm], marking or tagging individuals permanently has proved challenging. To date, long-term studies on individual dispersal and status change have not been possible. Using a combination of behavioural studies on marked individuals and genetic techniques (microsatellite markers), the above predictions about dispersal in *N. pulcher* were tested.

MATERIALS AND METHODS

STUDY AREA AND OBSERVATIONAL TECHNIQUES

The study site, on the Zambian shores of Lake Tanganyika, was in Kasakalawe Bay (8°46' S; 31°46' E), 100 m east of Kasakalawe village. Forty seven *N. pulcher* groups in 1997 (1 December 1996 to 9 April 1997) and 187 groups in 1998 (28 January to 7 May) were observed at this site. Only 38 of the original 47 groups were studied in 1998, as nine of the original groups had disappeared. In 1998, some new sub-populations with new groups were located in the study area (Table I). Observations were recorded on PVC slates while scuba diving to depths of 7–12 m (Balshine *et al.*, 2001). Repeated visits (at least twice a week during both field seasons) to all territories revealed the group composition, reproductive status (indicated by presence of young), and length of breeder tenure.

Individuals were identified as *N. pulcher* according to criteria give by Trewavas & Poll (1952), Poll (1974) and Colombe & Allgayer (1985). The fish have a very characteristic head colouration pattern. There are two vertical stripes behind the eye: one of them starts right behind the eye and curves down onto the preoperculum while the other is situated at the edge of the operculum and it runs past the operculum at the top. The study site is also situated in an area within the range distribution of *N. pulcher* (Konings, 1998).

Individuals were captured on their territory using hand nets and transparent PVC tubes and, after a brief handling period (1–3 min), were immediately released in their original territory. Fish captured were measured and sexed by examination of the genital papilla (Balshine-Earn *et al.*, 1998). A small fin tissue sample was cut from either the dorsal or anal fin and preserved in 95% ethanol for storage and transport. The tissue sample taken soon grew back and the fish appeared to be completely unaffected by the small clip in their fin. Fish were marked by injecting non-toxic acrylic paint subcutaneously or into scale pockets (Balshine-Earn *et al.*, 1998). These marks typically faded after a few weeks or months and also did not affect fish behaviour. The distances between territories and sub-populations (for use with F_{ST} calculations) were measured with underwater measuring tapes.

Recapture and dispersal data were obtained as a result of a series of behavioural experiments performed over a period of 2 years (Balshine-Earn *et al.*, 1998; Balshine *et al.*, 2001; Werner *et al.*, 2003). Fifty-seven of the 187 groups in 1998 were sampled genetically and thus could be examined for possible recaptures (Table I).

GENOTYPING

Tissue samples were placed in 500 μ l of an extraction buffer (25 mM EDTA, 75 mM NaCl, 10 mM Tris, 3 μ g proteinase K and 10% SDS), incubated for 2 h at 56° C and further incubated at 37° C overnight. Three extractions with organic solvents followed, the first with the same volume of Tris-saturated phenol, the second with phenol/chloroform in a

TABLE I. A summary of the total number of groups in each sub-population in the study site, the number of groups that were sampled, the number of fish that were sampled per sub-population and the number of fish successfully genotyped per sub-population

| Sub-population | Year(s) studied | Total number of groups | Number of groups sampled | Number of fish sampled | Number of fish typed |
|----------------|-----------------|------------------------|--------------------------|------------------------|----------------------|
| 1 | 1997 | 14 | 13 | 41 | 41 |
| | 1998 | 10 | 1 | 4 | 2 |
| 2 | 1997 | 9 | 9 | 34 | 27 |
| | 1998 | 11 | 2 | 5 | 3 |
| 3 | 1997 | 7 | 4 | 25 | 17 |
| | 1998 | 11 | 7 | 31 | 25 |
| 4 | 1997 | 20 | 1 | 4 | 1 |
| | 1998 | 20 | 20 | 91 | 84 |
| 5 | 1997 | 2 | 2 | 8 | 8 |
| | 1998 | 0 | 0 | 0 | – |
| 6 | 1997 | 6 | 6 | 23 | 18 |
| | 1998 | 5 | 5 | 29 | 22 |
| 7 | 1997 | 7 | 3 | 7 | 7 |
| | 1998 | 5 | 5 | 15 | 13 |
| A | 1998 | 10 | 5 | 18 | 16 |
| B | 1998 | 9 | 6 | 24 | 21 |
| C | 1998 | 3 | 1 | 6 | 6 |
| D | 1998 | 2 | 0 | – | – |
| F | 1998 | 18 | 0 | – | – |
| G | 1998 | 22 | 4 | 12 | 11 |
| H | 1998 | 17 | 0 | – | – |
| I | 1998 | 8 | 0 | – | – |
| J | 1998 | 5 | 0 | – | – |
| K | 1998 | 22 | 1 | 3 | 2 |
| L | 1998 | 9 | 0 | – | – |

In 1997, seven sub-populations were studied and 142 individuals had tissue collected, of which 119 were successful genetically. In 1998, the number of sub-populations sampled increased to 18 and a total of 238 fish had tissue taken for analysis, of which 205 were successfully analysed genetically.

1:1 ratio by volume, and the third with chloroform/isoamylalcohol in a 24:1 ratio by volume. The DNA was precipitated using two volumes of cold ethanol, and chilled at -20°C for 2 h prior to pelleting by centrifugation.

The five microsatellite loci used for genetic analysis are summarized in Table II. Loci were amplified in two $15\ \mu\text{l}$ reactions, using 40 ng of genomic DNA, 0.2 mM dNTPs, 0.5 μM of each primer (multiplexing), 1.5 μl $10\times$ polymerase chain reaction (PCR) buffer, 2.5 mM MgCl and 0.15 μl (0.75 units) Taq polymerase. PCR was carried out using a Perkin Elmer Geneamp 3600 and forward primers were labelled with fluorescent dyes. Initial denaturation occurred for 10 min at 94°C , followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 40 s and extension at 72°C for 70 s, followed by a final 1 h extension at 72°C . Formamide solution (40 μl) was combined with 4 μl of the PCR product before denaturation for 5–10 min at 98°C . The solution was loaded into an automatic capillary sequencer (ABI Prism 310 Genetic Analyser, Perkin Elmer). GENESCANTM500 TAMRA (red) (5 μl) was mixed with 150 μl formamide for use as a length standard. ABI's GENESCAN and GENOTYPER software (version 2.0) were used for gel analysis and genotyping.

TABLE II. Summary of the five microsatellite loci used in this study. Expected (H_E) and observed (H_O) heterozygosities as well as polymorphic information contents (PIC) were calculated using CERVUS version 2.0 (Marshall *et al.*, 1998). These values describe the estimated and actual allelic variation within a population and give a measure of how informative a given locus is (Strachan & Read, 2000). For the H_E , H_O and PIC a value of one is high and zero is low

| Locus | Reference | Number of alleles | H_E | H_O | PIC | Number of fish typed (%) |
|--------|--|-------------------|-------|-------|-------|--------------------------|
| 773 | Derived from <i>Tilapia cf. deckerti</i> . (Schliewen <i>et al.</i> , 2001) | 19 | 0.434 | 0.432 | 0.421 | 308 (95%) |
| 780 | Derived from <i>Tilapia cf. deckerti</i> . (Schliewen <i>et al.</i> , 2001) | 41 | 0.933 | 0.918 | 0.927 | 304 (94%) |
| 007 | Developed for <i>Neolamprologus multifasciatus</i> . (Kohler, 1997) | 8 | 0.449 | 0.446 | 0.430 | 303 (94%) |
| Pzeb3 | Developed for <i>Maylandia zebra</i> . (Parker & Kornfield, 1996) | 39 | 0.926 | 0.921 | 0.920 | 305 (94%) |
| LOC101 | Developed for <i>Lamprologus ocellatus</i> . (Brandtmann <i>et al.</i> , 1999) | 29 | 0.847 | 0.752 | 0.830 | 290 (90%) |

LOC101 was not in Hardy-Weinberg equilibrium. This heterozygote deficit, however, was found in only three of the 10 sub-populations (where >20% of individuals were genotyped).

Individuals were sorted by sex and size, and the allelic values generated from the peak values were compared between years to locate possible recaptured individuals. To assign recapture status, individuals were tested for identical alleles at all five loci for both years (1997 and 1998). An individual was included as a recapture if the alleles were identical at each available locus and the probability of such a match arising by chance was <1 in 1000. The likelihood of the individuals being genetically identical by chance was calculated by the formula: $[p_1q_1 \times p_2q_2 \times \dots \times p_nq_n]$, where n = number of loci typed, and p and q refer to the frequency of the specific allele at that locus (this formula is derived from the Hardy-Weinberg equilibrium; Griffiths *et al.*, 1996). Overall, the loci in this population were in equilibrium (Raymond & Rousset, 1995). In total, 119 fish from 1997 and 205 fish from 1998 were genotyped.

GENEPOP 3.1 (Raymond & Rousset, 1995) was used to determine allele frequencies at the five loci and to calculate F_{ST} measures between pairs of sub-populations (Aise, 1994). To ensure that F_{ST} calculations were representative of a sub-population, F_{ST} values were calculated for only those sub-populations in which $\geq 20\%$ of the individuals over 1.0 cm were sampled (the proportion of individuals within each sub-population that were genotyped at each locus ranged from 23 to 66%; Table III). The correlation between pair-wise F_{ST} estimates and physical distance was also estimated [following Rousset, 1997, the metric $F_{ST} (1 - F_{ST})^{-1}$ was used in place of F_{ST}].

To assess sex-biased dispersal further, the five microsatellite loci were analysed with the programme KINSHIP 1.3.1 (Goodnight *et al.*, 1997) to generate pair-wise relatedness values for every possible female and male breeder dyads. To reduce the effects of pseudoreplication (Knight *et al.*, 1999) the average relatedness of each individual to all other individuals of the same sex was calculated from the pair-wise values. A two-sample permutation test [RUNDOM Projects 1.1 (Jadwiszczack, 2002)] was then conducted to

TABLE III. Per cent of genetically sampled fish (by sub-population) in the F_{ST} analysis that were genotyped at each locus

| Year and sub-population | Number of fish | Per cent fish typed by locus | | | | | |
|-------------------------|----------------|------------------------------|-------|-------|-------|-------|---------|
| | | 773 | 780 | 007 | 003 | 101 | Average |
| 1997-1 | 41 | 100.0 | 97.6 | 97.6 | 95.1 | 87.8 | 95.6 |
| 1997-2 | 27 | 100.0 | 100.0 | 92.6 | 96.3 | 96.3 | 97.0 |
| 1997-3 | 17 | 100.0 | 94.1 | 94.1 | 94.1 | 94.1 | 95.3 |
| 1997-6 | 18 | 100.0 | 72.2 | 83.3 | 88.9 | 66.7 | 82.2 |
| 1998-3 | 25 | 88.0 | 92.0 | 88.0 | 92.0 | 96.0 | 91.2 |
| 1998-4 | 84 | 96.4 | 91.7 | 96.4 | 92.8 | 83.3 | 92.1 |
| 1998-6 | 22 | 100.0 | 100.0 | 95.5 | 90.9 | 95.5 | 96.4 |
| 1998-7 | 13 | 92.3 | 92.3 | 92.3 | 92.3 | 100.0 | 93.8 |
| 1998-A | 16 | 75.0 | 100.0 | 81.3 | 93.8 | 93.8 | 88.8 |
| 1998-B | 21 | 90.5 | 100.0 | 100.0 | 100.0 | 100.0 | 98.1 |

test for differences in average relatedness between breeding males and breeding females (Taylor *et al.*, 2003). Permutation tests draw random values from the population and assign them to the groups (in this case, dyads of breeding males and breeding females). The difference between the means of these simulated groups is then calculated. This procedure is repeated N times ($N=10000$ was used) and P values are obtained by comparing the frequency distribution of the permuted data to the difference between the two real group means. Similar analyses have been used to assess dispersal and genetic subdivision in other species, *e.g.* brown trout *Salmo trutta* L. (Carlsson *et al.*, 1999; Ruzzante *et al.*, 2001) and white shrimp *Litopenaeus setiferus* (Ball & Chapman, 2003).

Other statistical analyses were conducted using STATVIEW 5.0 or NTSYS-pc 1.8 (for $N \geq 20$). When needed ($N < 20$, in Appendix; Mundry & Fischer, 1998), the U statistic was calculated by hand (Siegel & Castellan, 1988). All statistics and P values were corrected for ties, and the P values presented are two-tailed. As tests were based on small sample sizes, *post hoc* power tests were conducted using the G*Power Program (Erdfelder *et al.*, 1996) to evaluate the strength of failing to reject the null hypothesis.

RESULTS

HOW MANY FISH DISPERSE AND WHERE DO THEY GO?

The F_{ST} values showed that there was little gene flow between sub-populations suggesting limited dispersal. Nearby sub-populations were more similar in their allele frequencies than more distant sub-populations (Mantel test, $n=9999$ permutations, $R=0.45$, $n=21$, $P=0.002$; Fig. 1). Also, both female and male breeders were more closely related to other same sex breeders within their sub-population than they were to same-sex breeders outside of their sub-population (two-sample permutation test, $n=10000$ randomizations, females: within sub-population $r=0.08 \pm 0.02$, outside sub-population $r=-0.04 \pm 0.01$, $P < 0.0001$; males: within sub-population $r=0.12 \pm 0.04$, outside sub-population $r=0.02 \pm 0.02$, $P=0.01$). Future studies are required to address more fine-scale patterns of genetic subdivision (comparing F_{ST} values calculated between groups within a sub-population to F_{ST} values calculated between sub-populations; see Table IV for preliminary data).

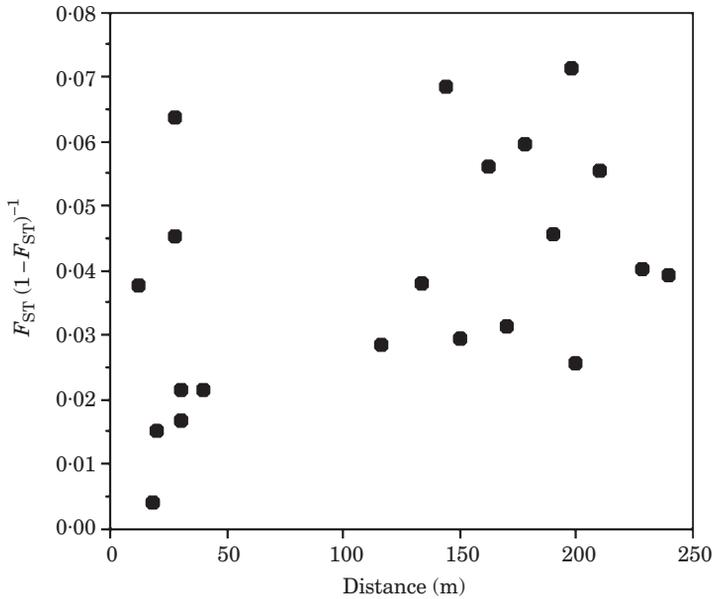


FIG. 1. The distance between sub-populations plotted in relation to $F_{ST} (1 - F_{ST})^{-1}$ values. The sub-populations included in this figure and the related analyses involved only those in which at least 20% of the individuals in the sub-population were genotyped.

Of the 119 fish from 1997 that were analysed genetically, 14 individuals (five females and nine males) were found to be present again in the sample of 205 genetically typed individuals from 1998. It should be noted that genetic sampling of individuals was not originally conducted for the purpose of identifying individuals present in both years. Genetic, sex and size data were used to confirm these recaptures; the probability that of any of these matches arose by chance is extremely small (Table V). Many possible statistics have been excluded in this section as the power of statistical tests carried out using these 14 individuals was low (power ranged from 0.054 to 0.501 based on the parametric equivalent of the non-parametric test; see Appendix for all statistics regarding these recaptured individuals).

The recapture data revealed that between years, six fish (43%) remained in their original territories, while eight (57%) dispersed to new territories. Of the eight fish that dispersed, five moved to adjacent territories, one to a non-adjacent territory, and two (both male helpers in 1997) moved to territories in different adjacent sub-populations 10 and 12 m away (Table V).

WHICH INDIVIDUALS DISPERSE?

Within a sub-population, there was no difference in relatedness between male dyads ($r = 0.12 \pm 0.04$) and female dyads ($r = 0.08 \pm 0.02$; two-sample permutation test, $n = 10\,000$ randomizations, $P = 0.29$). These results suggest that there may not be a sex bias in dispersal. At the population level, however, breeding males were more related to one another than were breeding females (all male dyads: $r = 0.03 \pm 0.017$, all female dyads: $r = -0.03 \pm 0.01$; $P = 0.004$),

TABLE IV. Comparison (for 10 sub-populations) of mean $F_{ST} \pm$ s.e. between groups (within a sub-population) and between sub-populations. Values are calculated using only those groups and sub-populations where >20% of the individuals were genotyped

| Year | Sub-population | Number of within | | Number of between | F_{ST} within sub-population | | F_{ST} between sub-populations |
|------|----------------|----------------------------|----------------------------|-------------------|--------------------------------|-------------------|----------------------------------|
| | | sub-population comparisons | sub-population comparisons | | (between groups) | (between groups) | |
| 1997 | 1 | 64 | | 3 | 0.062 \pm 0.010 | 0.030 \pm 0.012 | |
| 1997 | 2 | 28 | | 3 | 0.071 \pm 0.013 | 0.031 \pm 0.013 | |
| 1997 | 3 | 6 | | 3 | 0.064 \pm 0.020 | 0.038 \pm 0.008 | |
| 1997 | 6 | 6 | | 3 | 0.046 \pm 0.027 | 0.055 \pm 0.003 | |
| 1998 | 3 | 6 | | 5 | 0.022 \pm 0.008 | 0.030 \pm 0.004 | |
| 1998 | 4 | 120 | | 5 | 0.051 \pm 0.006 | 0.036 \pm 0.010 | |
| 1998 | 6 | 6 | | 5 | 0.031 \pm 0.016 | 0.056 \pm 0.007 | |
| 1998 | 7 | 6 | | 5 | 0.041 \pm 0.032 | 0.041 \pm 0.005 | |
| 1998 | A | 6 | | 5 | 0.001 \pm 0.001 | 0.029 \pm 0.007 | |
| 1998 | B | 15 | | 5 | 0.020 \pm 0.006 | 0.040 \pm 0.011 | |

TABLE V. Summary of the individuals designated as recaptured and the probability that each 'recapture' could represent two distinct individuals that share the same allele at each of the compared loci. As many loci (of the five possible) as could be resolved from the tissue were compared. The sex and growth data are consistent with the genetic data in each case

| | Sex | Year | Social position | L_s (cm) | Growth (cm year ⁻¹) | Sub-population | Distance travelled (cm) | Group | Chance of being different individual |
|----|--------|------|-----------------|------------|---------------------------------|----------------|-------------------------|------------------------|--------------------------------------|
| 1 | Female | 1997 | Helper | 4.9 | 0.10 | 2 | 102 | G | 3.8×10^{-08} |
| | | 1998 | Helper | 5.0 | | 2 | | C | |
| 2 | Female | 1997 | Helper | 4.9 | 0.10 | 6 | 0 | π | 1.1×10^{-04} |
| | | 1998 | Helper | 5.0 | | 6 | | π | |
| 3 | Female | 1997 | Helper | 5.6 | 0.00 | 3 | 0 | I | 1.1×10^{-07} |
| | | 1998 | Breeder | 5.6 | | 3 | | I | |
| 4 | Female | 1997 | Helper | 4.9 | 0.28 | 3 | 245 | III | 1.1×10^{-08} |
| | | 1998 | Breeder | 5.2 | | 3 | | II | |
| 5 | Female | 1997 | Helper | 5.1 | 0.11 | 3 | 56 | III | 1.1×10^{-09} |
| | | 1998 | Breeder | 5.2 | | 3 | | VIII | |
| 6 | Male | 1997 | Independent | 4.5 | 1.29 | 3 | 0 | III | 1.7×10^{-10} |
| | | 1998 | Helper | 5.7 | | 3 | | III | |
| 7 | Male | 1997 | Helper | 4.4 | 0.35 | 1 | 48 | 2 | 1.5×10^{-08} |
| | | 1998 | Helper | 4.8 | | 1 | | 4 | |
| 8 | Male | 1997 | Helper | 2.7 | 2.68 | 6 | 80 | π | 1.3×10^{-11} |
| | | 1998 | Helper | 5.2 | | 6 | | β | |
| 9 | Male | 1997 | Helper | 3.3 | 0.97 | 6 | 0 | γ | 2.2×10^{-06} |
| | | 1998 | Helper | 4.2 | | 6 | | γ | |
| 10 | Male | 1997 | Helper | 3.9 | 0.86 | 6 | 0 | π | 2.2×10^{-09} |
| | | 1998 | Helper | 4.7 | | 6 | | π | |
| 11 | Male | 1997 | Helper | 4.5 | 1.92 | 7 | 1200 | T | 9.3×10^{-06} |
| | | 1998 | Breeder | 6.2 | | 6 | | ϵ, β, π | |
| 12 | Male | 1997 | Helper | 5.3 | 0.54 | 1 | 1000 | 4 | 1.8×10^{-07} |
| | | 1998 | Breeder | 5.8 | | 4 | | 21 | |
| 13 | Male | 1997 | Breeder | 5.6 | 0.21 | 6 | 52 | δ | 1.4×10^{-07} |
| | | 1998 | Breeder | 5.8 | | 6 | | γ | |
| 14 | Male | 1997 | Breeder | 5.6 | 0.70 | 7 | 0 | X | 2.3×10^{-10} |
| | | 1998 | Breeder | 6.3 | | 7 | | X, T, U, Y, Z | |

suggesting that males typically disperse. The recapture data also suggested that both males and females dispersed; no sex differences in dispersal were detected using this method of recapture (Appendix). In total, one breeder (male) and seven non-breeders (three female and four male helpers) dispersed or changed territories between years (Table V). Dispersing male helpers ($n=4$) appeared to move further than dispersing female helpers ($n=3$) (medians: 540 *v.* 102 cm).

Repeated visits to each territory throughout the field season revealed that *c.* 12% of male breeders were naturally replaced every 50 days compared to only 5% of female breeders. This suggests that males tend to be replaced faster than females ($\chi^2 = 3.78$, $N_M = 17/127$, $N_F = 11/206$, $0.10 > P > 0.05$).

Dispersal does not seem to occur at a particular life stage in *N. pulcher*. The largest dispersing fish was 5.6 cm L_S in 1997 and had reached 5.8 cm in 1998. The smallest fish that dispersed was 2.7 cm in 1997 and had grown to 5.2 cm by 1998 (Table V). There was no initial size difference between fish that dispersed or stayed, even when males and females were compared separately (Appendix).

WHY DO INDIVIDUALS DISPERSE?

Individuals that bred in 1998 appeared to disperse further ($n=5$, median distance = 245 cm; range: 0–1200 cm) than those that did not ($n=7$, median distance = 0 cm, range: 0–102 cm). Female helpers who achieved a breeding position by 1998 ($n=3$) travelled a median distance of 56 cm (range 0–245 cm), while male helpers becoming breeders in 1998 ($n=2$) travelled 1100 cm (range: 1000–1200 cm). Indeed, the only individuals that moved long distances (between sub-populations) were the two helper males that became breeders in 1998.

Six of the seven helpers that dispersed increased their status (by becoming a breeder or by moving up in the dominance hierarchy). The one helper whose position decreased moved from a group where it was the only helper (group size = 3) to a group of eight where it was second in the helper dominance hierarchy.

STATUS DYNAMICS

The probability of male and female helpers becoming breeders was similar (3/5 females *v.* 2/7 males; Fisher's exact test, $n=12$, $P=0.27$, Power = 0.13). Three of the five recaptured females (all helpers in 1997) became breeders in 1998; two remained in helper status. Six of the nine recaptured males retained their 1997 positions in 1998; the two male breeders were again found to be breeders and four male helpers remained in their previous helper status. One of the male breeders expanded his range from breeder of only one territory (1997) to five territories (1998). Two male helpers became breeders. One male, a group independent who was accepted by a group but did not help in 1997, was found to be an active helper in that group in 1998.

One female (a helper in 1997) became a breeder in her previous territory in 1998, confirming results from an experimental removal study that showed that helpers can take over in the territory where they had previously helped (Balshine-Earn *et al.*, 1998). Unfortunately, it remains unknown whether this female took over because a vacancy arose (*via* breeder death) or as a result of a successful challenge of the previous breeder.

DISCUSSION

The positive relationship between F_{ST} and physical distance, coupled with the fact that male and female breeders were more closely related to breeders (of the same sex) from their own sub-population than to breeders from other sub-populations, supports the notion that there is limited dispersal in *N. pulcher*. As expected, *N. pulcher* dispersal better fits a stepping stone (Kimura, 1994) than an infinite island (Wright, 1931, 1943) model of dispersal. This finding is also significant in that it shows a genetic division of sub-populations over a relatively small geographical area (maximum distance between sub-populations was 228 m²). Selander (1970) found similar small-scale genetic structuring in wild populations of house mice *Mus musculus*; several genetically distinct tribes of mice existed within a single barn structure.

The present study did not show conclusive evidence of sex-biased dispersal in *N. pulcher*. Males and females were equally related within a sub-population and the recapture data showed no overall difference in dispersal rates or distance by males *v.* females. At the population level, however, male dyads were more closely related compared to female dyads suggesting that males may disperse farther. Also, the only individuals that travelled between sub-populations were two male helpers who became breeders, suggesting that males may disperse further. Alternatively, the population results of more related males may have arisen because females actually disperse further (perhaps outside of the sampled study area). Admittedly, the small number of recaptured individuals and the conflicting recapture and genetic results weakens the ability to strongly ascertain which sex is dispersing.

Dispersal patterns in other co-operatively breeding species are known. For example, Florida scrub jay females tend to disperse earlier and further than males, as females almost always become breeders by taking an available breeding spot in a different territory, while male scrub jays usually inherit all or a portion of their natal territory (Woolfenden & Fitzpatrick, 1990). Seychelles warblers delay dispersal until they can move to a territory of equal or higher quality to their natal territory (Hatchwell & Komdeur, 2000) and show a sex difference in dispersal and breeding strategies. Female Seychelles warblers help and occasionally breed on their natal territory and may disperse to a new territory to gain a breeding position, while males tend to 'bud-off' a portion of their natal territory to breed in (Komdeur & Edelaar, 2001). Dwarf mongooses may become breeders in one of two ways: subordinates may disperse to a new pack, or remain in their natal pack and attain dominance and therefore breeder status. Although both sexes disperse in mongoose, males disperse more frequently and farther than females (Creel, 1994). This study shows that there are two strategies to become a breeder in *N. pulcher*: territory inheritance and dispersing to fill a breeding vacancy. Further studies on dispersal are now planned to examine whether *N. pulcher* has sex-biased dispersal and whether the pattern is more similar to the female-biased dispersal of the Florida scrub-jay, or the more male-biased pattern of dwarf mongoose.

The results hint at the possibility that individuals disperse in order to gain a better position in the group. The idea of 'trading-up' in the hierarchy appears to be reflected in those individuals that dispersed; six of seven helpers were

observed to move into a higher dominance position in their new group. The possibility that the dispersal occurred as a result of eviction from their former group, however, cannot be eliminated without further study. If helpers move voluntarily between breeding groups, breeders may have to compete for helpers and offer reproductive incentives to bribe helpers to join their group (Reeve, 1998). The helpers that changed groups but remained in helper status often moved what would appear to be extremely small distances. As this species has an extremely small substratum-based home range (Balshine *et al.*, 2001; Werner *et al.*, 2003), however, these small movements constitute true dispersal events.

Another interesting social change was the group independent male in 1997 who became an active group helper in 1998. This individual held an independent shelter on the edge of a group's territory and provided no help in 1997; in 1998 he was strongly associated with this group and provided extensive help. In laboratory experiments using *Neolamprologus brichardi* (Poll), Taborsky (1985) showed that expelled helpers may be re-accepted in their group if the need for help (defence against a conspecific or space competitor) was increased. In the case observed in this study, the reasons for the change in helping effort and new association cannot be directly determined; the breeders in this group were not genetically sampled in 1998 so this helper's relatedness to breeders cannot be compared between the 2 years. It is possible that increased helping behaviour may be a method of securing better resources, such as shelter (Balshine *et al.*, 2001), or a way to move up into a higher, more dominant social position in preparation for future inheritance of a breeding vacancy (Carlisle & Zahavi, 1986; Balshine-Earn *et al.*, 1998; Lotem *et al.*, 1999).

These results provide a useful first step in understanding social change and dispersal patterns in a co-operatively breeding fish. They show that genetic analyses can be used to assess life-history variables such as growth, survival and reproductive success. Additional work is now needed to determine precisely how dispersal varies with sex and size of individual, and further explore the relationship between status change and dispersal and 'shed light' on the factors driving dispersal patterns in *N. pulcher*.

We thank B. Leach, F. Neat, H. Reid and N. Werner for field assistance. A. Kuntz and M. Maan also assisted in collecting some tissue samples. R. Achmann helped with the genetic techniques and H.L. Gibbs and J.S. Quinn provided direction and advice for the genetic data analysis. In addition, we thank D. Ruzzante, two anonymous reviewers, E. Bressler, V. Chant, M. Daly, D. Earn, J. Galef, N. Goodwin, D. Hart, S. Houshmandi, S. Marsh, F. Neat and K. Sloman for helpful comments on the manuscript. The work was funded by grants from Fonds zur Förderung der wissenschaftlichen Forschung (P 10916-Bio) to MT; and a NSERC research grant and a Royal Society of London fellowship to SB. This research was conducted under the guidelines of the Research and Ethics Board of McMaster University and with the permission and co-operation of the Leonard Mwape and the Zambian Ministry of Agriculture, Food and Fisheries.

References

- Andersson, M. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.
Avice, J. C. (1994). *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall.

- Ball, A. O. & Chapman, R. W. (2003). Population genetic analysis of white shrimp, *Litopenaeus setiferus*, using microsatellite genetic markers. *Molecular Ecology* **12**, 2319–2330.
- Balshine, S., Leach, B., Neat, F., Reid, H., Taborsky, M. & Werner, N. (2001). Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). *Behavioral Ecology and Sociobiology* **50**, 134–140.
- Balshine-Earn, S., Neat, F. C., Reid, H. & Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behaviour in a cooperatively breeding fish. *Behavioral Ecology* **9**, 432–438.
- Brandtmann, G., Scandura, M. & Trillmich, F. (1999). Female–female conflict in the harem of a snail cichlid (*Lamprologus ocellatus*): behavioural interactions and fitness consequences. *Behaviour* **136**, 1123–1144.
- Carlisle, T. R. & Zahavi, A. (1986). Helping at the nest, allofeeding and social status in immature Arabian babblers. *Behavioral Ecology and Sociobiology* **18**, 339–351.
- Carlsson, J., Olsén, K. H., Nilsson, J., Øverli, Ø. & Stabell O. B. (1999). Microsatellites reveal fine-scale genetic structure in stream-living brown trout. *Journal of Fish Biology* **55**, 1290–1303. doi: 10.1006/jfbi.1999.1123.
- Colombe, J. & Allgayer, R. (1985). Description de Variabilichromis, *Neolamprologus* et *Palaeolamprologus*, genres nouveaux du lac Tanganika, avec redescription des genres *Lamprologus* (Schilthuis, 1891) et *Lepidiolamprologus* (Pellegrin, 1904). *Revue Française des Cichlidophiles* **49**, 9–28.
- Creel, S. R. (1994). Inclusive fitness and reproductive strategies in dwarf mongooses. *Behavioral Ecology* **5**, 339–348.
- Erdfelder, E., Faul, F. & Buchner, A. (1996). GPOWER: a general power analysis program. *Behavior Research Methods, Instruments & Computers* **28**, 1–11.
- Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* **26**, 645–652.
- Griffiths, A. J. F., Miller, J. H., Suzuki, D. T., Lewontin, R. C. & Gelbart, W. M. (1996). *An Introduction to Genetic Analysis*, 6th edn. New York: W.H. Freeman and Company.
- Hatchwell, B. J. & Komdeur, J. (2000). Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour* **59**, 1079–1086.
- Irwin, A. J. & Taylor, P. D. (2000). Evolution of dispersal in a stepping-stone population with overlapping generations. *Theoretical Population Biology* **58**, 321–328.
- Kimura, M. (1994). “Stepping-stone” model of population. In *Population Genetics, Molecular Evolution, and the Neutral Theory: Selected Papers* (Kimura, M., ed.), pp. 133. Chicago, IL: University of Chicago Press.
- Knight, M. E., Turner, G. F., van Oppen, M. J. H., Rico, C. & Hewitt, G. M. (1999). Microsatellite DNA and sex-biased dispersal among Lake Malawi cichlids. *Molecular Ecology* **8**, 1521–1527.
- Kohler, U. (1997). Zur Struktur und Evolution des Sozialsystems von *Neolamprologus multifasciatus* (Cichlidae, Pisces), des kleinsten Schneckenbuntbarsches des Tanganjika-Sees. PhD Thesis, Ludwig-Maximilians-Universität, München.
- Komdeur, J. & Edelaar, P. (2001). Evidence that helping at the nest does not result in territory inheritance in the Seychelles warbler. *Proceedings of the Royal Society of London, Series B* **268**, 2007–2012.
- Konings, A. (1998). *Tanganyika Cichlids in their Natural Habitat*. St Leon-Rot: Cichlid Press.
- Lotem, A., Wagner, R. H. & Balshine-Earn, S. (1999). The overlooked signalling component of non-signalling behavior. *Behavioral Ecology* **10**, 209–212.
- Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**, 639–655.
- Mundry, R. & Fischer, J. (1998). Use of statistical programs for non-parametric tests of small samples often leads to incorrect *P* values: examples from *Animal Behaviour*. *Animal Behaviour* **56**, 256–259.

- Parker, A. & Kornfield, I. (1996). Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes* **47**, 345–352.
- Poll, M. (1974). Contribution à la faune ichthyologique du lac Tanganika d'après les récoltes de *P. brichard*. *Revue de Zoologie africaine* **88**, 99–110.
- Pusey, A. E. (1987). Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution* **2**, 295–299.
- Raymond, M. & Rousset, R. (1995). GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.
- Reeve, H. K. (1998). Game theory, reproductive skew and nepotism. In *Game Theory and Animal Behaviour* (Dugatkin, L. & Reeve, H. K., eds), pp. 118–145. Oxford: Oxford University Press.
- Rousset, F. (1997). Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**, 1219–1228.
- Ruzzante, D. E., Hansen, M. M. & Meldrup, D. (2001). Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. *Molecular Ecology* **10**, 2107–2128.
- Schliwien, U. K., Rassmann, K., Markmann, M., Markert, J., Kocher, T. D. & Tautz, D. (2001). Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. *Molecular Ecology* **10**, 1471–1488.
- Selander, R. K. (1970). Behavior and genetic variation in natural populations. *American Zoologist* **10**, 53–66.
- Siegel, S. & Castellan, N. J., Jr. (1988). *Nonparametric Statistics for the Behavioral Sciences*, 2nd edn. New York: McGraw Hill.
- Strachan, T. & Read, A. P. (2000). *Human Molecular Genetics*, 2nd edn. New York: Wiley-Liss.
- Taborsky, M. (1984). Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. *Animal Behaviour* **32**, 1236–1252.
- Taborsky, M. (1985). Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. *Behaviour* **95**, 45–75.
- Taborsky, M. & Grantner, A. (1998). Behavioural time-energy budgets of cooperatively breeding *Neolamprologus pulcher* (Pisces: Cichlidae). *Animal Behaviour* **56**, 1374–1382.
- Taborsky, M. & Limberger, D. (1981). Helpers in fish. *Behavioral Ecology and Sociobiology* **8**, 143–145.
- Taylor, M. I., Morley, J. I., Rico, C. & Balshine, S. (2003). Evidence for genetic monogamy and female-biased dispersal in the biparental mouthbrooding cichlid *Eretmodus cyanostictus* from Lake Tanganyika. *Molecular Ecology* **12**, 3173–3177.
- Taylor, P. D., Irwin, A. J. & Day, T. (2000). Inclusive Fitness in finite deme-structured and stepping-stone populations. *Selection* **1**, 153–163.
- Trewavas, E. & Poll, M. (1952). Three new species and two new sub-species of the genus *Lamprologus*, cichlid fishes of Lake Tanganyika. *Proceedings of the Royal Society of London B* **116**, 240–246.
- Werner, N. Y., Balshine-Earn, S., Leach, B. & Lotem, A. (2003). Helping opportunities and space segregation among helpers in cooperatively breeding cichlids. *Behavioral Ecology* **14**, 749–756.
- Woollfenden, G. E. & Fitzpatrick, J. W. (1990). Florida Scrub Jays: a synopsis after 18 years of study. In *Cooperative Breeding in Birds: Long-term Studies of Ecology and Behavior* (Stacey, P. B. & Koenig, W. D., eds), pp. 241–266. New York: Cambridge University Press.
- Wright, S. (1931). Evolution in Mendelian populations. *Genetics* **16**, 97–159.
- Wright, S. (1943). Isolation by distance. *Genetics* **28**, 114–138.

Electronic References

- Goodnight, K. F., Queller, D. C. & Poznansky, T. (1997). *KINSHIP 1.3.1*. Available at: <http://gsoftnet.us/GSoft.html>.
- Jadwiszczack, P. (2002). *RUNDOM Projects 1.1*. Available at: <http://pjadw.tripod.com/rsite.htm>.

APPENDIX. Statistics of the 14 recaptured individuals

| | Mann-Whitney <i>U</i> -test | Power |
|---|-----------------------------|-------|
| A. Size (L_S , cm in 1997) and dispersal | | |
| Dispersers ($N=8$, median = 4.9). | $U = 24.5$ | 0.054 |
| v. stayers ($N=6$, median = 4.7). | $P > 0.20$ | |
| Male dispersers ($N=4$, median = 4.5). | $U = 7.5$ | 0.065 |
| v. male stayers ($N=3$, median = 3.9)*. | $P > 0.20$ | |
| Female dispersers ($N=3$, median = 4.9). | $U = 4$ | 0.083 |
| v. female stayers ($N=2$, median = 5.2)*. | $P > 0.20$ | |
| B. Sex and dispersal | | |
| Males ($N=9$) | $U = 24$ | 0.118 |
| v. females ($N=5$). | $P > 0.20$ | |
| Male dispersers ($N=4$) | $U = 7$ | 0.207 |
| v. female dispersers ($N=3$)*. | $P > 0.20$ | |
| C. Social changes and dispersal | | |
| Individuals that became breeders ($N=5$) | $U = 28$ | 0.411 |
| v. individuals that did not ($N=7$)*. | $0.20 > P > 0.10$ | |
| Males that became breeders ($N=2$) | $U = 10$ | 0.501 |
| v. males that did not ($N=5$)*. | $P = 0.10$ | |
| Females that became breeders ($N=3$) | $U = 3.5$ | 0.067 |
| v. females that did not ($N=2$)*. | $P > 0.20$ | |

*These comparisons involve only those individuals who were non-breeders in 1997.