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GENETIC PARENTAGE AND VARIABLE SOCIAL STRUCTURE IN BREEDING SOUTHERN LAPWINGS

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Abstract. We examined the genetic mating system and social breeding structure of the Southern Lapwing (*Vanellus chilensis*), a shorebird that breeds in Brazil in secluded pairs or groups (3–4 adults). We never observed copulation between males or females of primary pairs with additional adults in groups; all recorded copulations were between primary pair members. Where DNA from primary males was available, we found mixed paternity in 18.8% of the broods (3 of 16), comprising 9.8% of the chicks (4 of 41). All broods containing young sired by different males occurred in groups. One chick had a DNA fingerprint with four novel bands but shared a high proportion of bands with both primary pair members of its group. One explanation is that the chick's mother copulated with a relative of her social mate. However, quasi parasitism (i.e., nest-sharing by two females) cannot be excluded. Our study demonstrates a flexible social and genetic mating system in Southern Lapwings.

Key words: *mating system, parentage, paternity, sociality, Southern Lapwing*, Vanellus chilensis.

Paternidade Genética e Estrutura Social Variável na Reprodução de *Vanellus chilensis*

Resumo. Estudamos o sistema genético de acasalamento e estrutura reprodutiva social de *Vanellus chilensis*, espécie que reproduz-se no Brasil em pares ou em grupos (3–4 adultos). Nunca observamos cópula entre o macho ou a fêmea do par primário com adultos extra; todas as cópulas registradas ocorreram entre membros do par primário. Quando o DNA do macho primário foi amostrado, encontramos evidência de paternidade compartilhada em 18,8% das proles (3 de 16), compreendendo 9,8% dos filhotes (4 de 41). Todas as ninhadas contendo filhotes oriundos de pais diferentes ocorreram nos grupos reprodutivos. Em um caso houve um ninhego com quatro novas bandas, mas que compartilhava uma alta proporção de bandas com ambos membros do par primário. Uma possível explicação é que a mãe deste filhote teria acasalado com um parente do macho social. No entanto, a possibilidade do quasi-parasitismo (i.e., ninho partilhado por duas fêmeas) não pode ser excluída. Nosso estudo evidencia a flexibilidade social e genética do sistema de acasalamento de *V. chilensis*.

Cooperative breeding is a type of social organization characterized by the presence of sexually mature individuals, in addition to the primary breeding pair, that assist in caring for the offspring (equivalent to communal breeding, as defined by Brown [1987]). In birds and mammals, these extra individuals (helpers) can be family members, usually previous offspring of the breeding pair that remain in their natal groups or territories (Emlen 1995). Alternatively, the helpers may be adults that do not necessarily abstain from reproduction, thus leading to the sharing of reproductive opportunities within the group (Stacey and Koenig 1990). Cooperative breeding is relatively rare among vertebrates, known for only about 3% of bird and mammal species (Emlen 1997).

Plovers (Charadriidae) are precocial, ground-dwelling birds that exhibit variable mating patterns and flexible social structures. Their social mating systems include monogamy, polygyny and polyandry (Lessells 1984, Byrkjedal et al. 1997, Parish et al. 1997), and parental care ranges from biparental care to uniparental care by either sex (Blomqvist and Johansson 1994, Liker and Székely 1997, 1999). Individuals defend territories either as secluded pairs (two adults) or, more rarely, in groups (>2 adults). The latter has been observed in Southern Lapwings (*Vanellus chilensis*), one of the least-known species in the family. This widespread plover, occurring from Central America to the southernmost tip of South America, breeds in open habitats with short vegetation, including pastures, river plains, and plowed lands (Sick 1997). Southern Lapwings can breed twice or occasionally three times per breeding season, producing, clutches averaging three eggs (range: 2–4) in size (Walters 1982). In central Brazil (where this study was conducted), lapwings breed as secluded pairs or in groups of three to four birds that aggressively defend territories. In the literature, Southern Lapwings have been described as pair breeding and monogamous (Ligon and Burt 2004) as well as cooperatively breeding (Walters and Walters 1980).

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Our study, using DNA fingerprinting, investigated the social and genetic mating system of Southern Lapwings. In particular, we examined whether deviations from genetic monogamy were more likely in breeding groups than in secluded territorial pairs, which could indicate shared breeding in groups.

METHODS

STUDY AREA

We studied 36 breeding units (pairs or groups) of Southern Lapwings in two areas in central Brazil located approximately 40 km apart (15°46′S, 47°52′W and 15°56′S, 47°56′W), from July 2000 to June 2002. These units attempted breeding a total of 98 times, 15 of which failed due to predation. We recorded multiple breeding attempts by some units over different seasons and also within seasons, but in the latter case only when an earlier breeding attempt failed due to predation. The study areas were similar and consisted typically of altered savanna combined with gardens and fields within a suburban matrix around the city of Brasilia. The breeding territories were scattered among fields and lawns, and were separated by houses, roads, and buildings. Mean territory size was approximately 3–4 ha, with no significant difference between secluded pairs and groups (data from 30 breeding units; RHM, unpubl. data). In central Brazil, lapwings breed from July to December, a period that encompasses part of the dry season (between May and September) and the early wet season.

CAPTURE OF BIRDS

We captured adults during nesting using fall-traps (mesh boxes, 40 × 40 cm) positioned over nests, and also with nylon nooses placed around nests (staked to the ground to prevent flight), which were then tightened from a distance around adults' legs. We recorded four cases of permanent nest desertion after trapping. In all other cases, the birds resumed incubation within minutes after being released. We marked 49 adults individually with colored plastic and metal bands (Centro Nacional de Pesquisa para Conservação das Aves Silvestres, Brazil). Some of these adults (44 birds from 32 breeding units) were recorded breeding during more than one season: 18 were observed in two seasons and 26 in three seasons. Adult Southern Lapwings are sexually monochromatic, and we also noticed no obvious external biometric differences between the sexes (RHM, unpubl. data). We collected blood samples from adults (25–50 µl) by puncturing the brachial vein. Blood samples from chicks (about 25 µl) were collected by puncturing the metatarsal vein.

In groups, the identity of the primary pair members (which we designated as the putative parents) and additional adults was determined based on the fact that usually only two individuals risked incubating eggs after traps were set over the nests. Therefore, we were able to obtain blood samples from only these birds. The extra adults occasionally incubated for short periods, but never with a trap in place. Degree of parental care may reflect the certainty of genetic representation in the offspring (Neff and Gross 2001). We therefore assumed that the two individuals (primary pair) within groups that risked incubation when traps were set were putative parents, as their behavior may have reflected a higher confidence of genetic parentage.

SOCIAL STRUCTURE

During the reproductive period, the territories of the 36 breeding units were visited every two to three days between 05:30 and 18:30 (GMT–3), to check for the presence of birds. The primary pair members were usually color banded (49 of 72 individuals), but additional adults were unmarked. Observations were made on each territory for a minimum of 10 min if all birds of the pair or group were present. If none or only part of the group was present, we waited up to 30 min to ascertain territorial occupation and group composition. During each visit, we noted banded and unbanded birds in the territory, presence of a nest, number of eggs or chicks, behavior (including copulation), and anthropogenic changes to the area. Territories remained stable throughout the study period in terms of social structure (secluded pair versus group).

MULTILOCUS DNA FINGERPRINTING

We examined genetic parentage with multilocus DNA fingerprinting, following standard laboratory and scoring procedures (Westneat 1993, Krokene et al. 1996). In brief, genomic DNA was extracted from blood samples using proteinase K, phenol-chloroform (70:30) and chloroform-isoamyl alcohol (24:1). We loaded 3–5 µg of HaeIII-digested DNA onto 20 × 40 cm 0.8% agarose gels in 1x TBE buffer. To optimize comparability of presumed parents and their young, we placed all samples from a breeding unit on the same gel, with the DNA from the chicks flanking the samples from the primary pair members. When samples were available from multiple broods tended by the same primary pair, DNA from all of their chicks were loaded on the same gel, repeating the parental samples 2–3 times. The number of analyzed chicks per pair or group therefore varied between one and eight. After electrophoresis at 1.2 V per cm for 40 hr, the DNA was transferred onto nylon membranes using Southern blotting and hybridized with the multilocus probe *per* (Shin et al. 1985). The probe was radioactively labeled with [^{32}P]dCTP by random priming using the Prime-a-Gene labeling system (Promega, Mannheim, Germany). Membranes were autoradiographed with one intensifying screen at –70°C for 3–8 days.

Given the available blood samples, we fingerprinted parents and offspring from 20 pairs or groups, thus representing a subset of the 36 breeding units monitored. In total, we analyzed 25 adults and 59 chicks from 23 broods, 3 of which were multiple breeding attempts by the same primary pair (two pairs and one group). About half of the broods (12) were tended by secluded pairs and half (11) by breeding groups. Blood samples for nine families (three pairs, six groups) were obtained from both members of the primary pair and their chicks ($n = 29$); for five families (three pairs, two groups), we obtained blood samples from only the primary male and chicks ($n = 12$); and for two families (one pair, one group) we obtained blood samples from only the primary female and chicks ($n = 6$). In the remaining four families (three pairs, one group; 12 chicks), none of the primary pair members were available for blood sampling. All fingerprints were scored by VS. On average, 15.7 bands were scored in adults (SE = 1.0, range: 9–28, $n = 25$) and 16.7 bands in chicks (SE = 0.7, range: 6–33, $n = 59$).

When both members of a primary pair were available, we determined parentage using number of novel bands (chick bands that cannot be attributed to either of the pair members) and band sharing (the proportion of bands shared between two individuals). Assuming that 15 chicks with no novel bands were genetic offspring of the primary pairs of their respective nests, and that one novel band in each of four chicks was due to mutations or other random causes (Westneat 1993), the probability per chick of finding k novel bands due to random causes alone was $(4/19)^k$. Thus, the probability of finding two or three novel bands in a

genetic offspring was 0.044 and 0.009, respectively. In our sample of 29 chicks (with both primary pair members available), we thus expected fewer than one young with three novel bands, and we therefore set the exclusion criterion to more than two novel bands. The band-sharing coefficient (D) was calculated as described by Wetton et al. (1987). As the expected lower limit of band sharing between chicks and their genetic parents, we used the lowest observed band sharing between parents and chicks, considering only chicks with zero novel bands ($D = 0.32$). The latter value coincided with the lower, one-tailed 95% distribution limit for band sharing between parents and chicks with zero novel bands. We therefore used D-values below 0.32 for excluding parentage when only one of the members of the primary pair was available. Finally, when the primary male was not available, we checked for indications of mixed paternity by examining band sharing among brood mates (excluding one family containing the female and only one chick), expecting half-siblings to show D-values around 0.25. Given that the lower, one-tailed 95% distribution limit for band sharing between parents and chicks with zero novel bands (i.e., genetic offspring) was 0.32, first-order relatives, including full siblings, should show band-sharing coefficients above 0.31. In the five families where we examined band sharing between brood mates, mean D-values varied between 0.36 and 0.51 (see below), suggesting that these chicks were full siblings. Band sharing between pair members (i.e., presumably unrelated individuals), varied between 0 and 0.14 (mean ± SE: 0.06 ± 0.02, $n = 9$ pairs). The upper, one-tailed 95% distribution limit for band sharing between these adults was 0.17.

MOLECULAR SEXING

Following the method described below, we determined the sex of 24 of the adults included in the parentage analysis (one individual was excluded due to an insufficient amount of DNA) and of 3 additional adults not included in the parentage analysis. In total, the molecular sexing successfully identified 16 males and 11 females. Ten of these individuals were initially sexed based on their behavior (i.e., copulatory position), which was in all cases confirmed by the molecular sexing.

We used a well-established molecular technique (Fridolfsson and Ellegren 1999) that is based on the amplification of a CHD1 gene intron, located on the avian sex chromosomes. This intronic sequence shows a consistent size difference between the two sex chromosomes; males can therefore be identified by a single CHD1Z product, whereas females usually show two amplified fragments corresponding to CHD1Z and CHD1W (Fridolfsson and Ellegren 1999).

We first determined the sex-specific fragment patterns by analyzing the DNA of two presumed males and females (based on behavioral observations during copulation), following the protocol used by Fridolfsson and Ellegren (1999). In brief, 10 µl PCRs contained approximately 100 ng genomic DNA, final concentrations of 200 µM dNTPs and 1.75mM $MgCl_2$, 2 pmol of primers 2550F and 2718R (Fridolfsson and Ellegren 1999) and 1.0 U Taq polymerase (Promega, Mannheim, Germany). Amplification was carried out on a T1 thermocycler (Biometra, Goettingen, Germany) and consisted of an initial denaturation step at 94°C for 2 min, followed by a 10-cycle touchdown profile (94° C for 30 sec, 60°C–50°C for 30 sec, 72°C for 40 sec), 30 cycles with a constant annealing temperature (94°C for 30 sec, 50°C for 30 sec, 72°C for 40 sec), and ending with a final extension step at 72°C for 5 min. The sizes of the PCR products were determined by agarose gel electrophoresis. The presumed males and females were unequivocally identified by the expected, sex-specific banding pattern (Fridolfsson and Ellegren 1999). Various control amplifications (single primer controls and reactions lacking either both primers or DNA) confirmed reaction specificity to the CHD1 sequence.

All data were analyzed using SYSTAT 9 (SPSS 1999). Unless otherwise indicated, we report means ± SE throughout the paper.

RESULTS

GENETIC PARENTAGE

In the nine complete families sampled (both primary pair members available), about half of the chicks (15 of 29) had fingerprints fully matching those of the primary pair (i.e., they showed no novel bands; Fig. 1). Mean band sharing with the primary female was 0.49 ± 0.03 (range: 0.33–0.78) and with the primary male 0.51 ± 0.03 (range: 0.32–0.74). The fingerprints of ten chicks each revealed 1–2 novel bands (Fig. 1). With one exception, these chicks also shared a high proportion of bands with the primary pair members of their units (mother-offspring 0.53 ± 0.02, range: 0.45–0.61; father-offspring 0.48 ± 0.03, range: 0.38–0.65). The exception was a chick with one novel band that shared many bands with the primary female ($D = 0.67$) but few with the male ($D = 0.21$; Fig. 1). Since the latter may have been caused by a marked difference in strength between the two fingerprints (the male's profile was much stronger), we conservatively assumed that this chick also had been sired by the primary male of its unit. We conclude that all chicks with 0–2 novel bands were the genetic offspring of their units' primary pairs.

The fingerprints of the remaining four chicks (from three broods that contained two, three and five chicks, respectively) each contained 3–8 novel bands. Three of these chicks showed relatively high band sharing with the primary female ($D \geq 0.40$) and low band sharing with the primary male ($D \leq 0.25$; Fig. 1) of their respective units. We therefore concluded that they were each sired by a male other than the primary male. A fourth chick with four novel bands shared a relatively high proportion of bands with both members of the primary pair (D mother = 0.40, D father = 0.38) of its unit. Given the low probability of mutations or other random factors resulting in four novel bands ($P = 0.002$), we classified this chick as a genetic mismatch with either or both members of the primary pair (Fig. 1).

When only one member of a primary pair was available, band-sharing values suggested that all chicks were the genetic offspring of the tending parent in question. These chicks thus shared on average 52% of the bands with the primary female (SE = 0.04, range: 0.43–0.70, $n = 6$ chicks) or 49% with the primary male (SE = 0.02, range: 0.38–0.60, $n = 12$ chicks). Finally, band-sharing values among brood mates revealed no strong indications of mixed paternity in one of the female-only families (mean $D = 0.51$), or in the four families where none of the primary pair members were available (mean D varying between 0.36 and 0.42).

Considering only the families from which DNA from the primary male (presumed father) was available, we found evidence of paternal genetic mismatches in 18.8% of the broods (3 of 16), comprising 9.8% of the chicks (4 of 41). An additional six broods, containing 17 chicks, revealed no indications of mixed paternity.

COPULATION AND GENETIC MISMATCHES IN RELATION TO SOCIAL STRUCTURE

Copulation occurred both before as well as after clutches were laid. We observed a total of 48 copulations between banded individuals in 21 breeding units (groups or pairs), all of which were between the male and female of a primary pair. Thus, we never

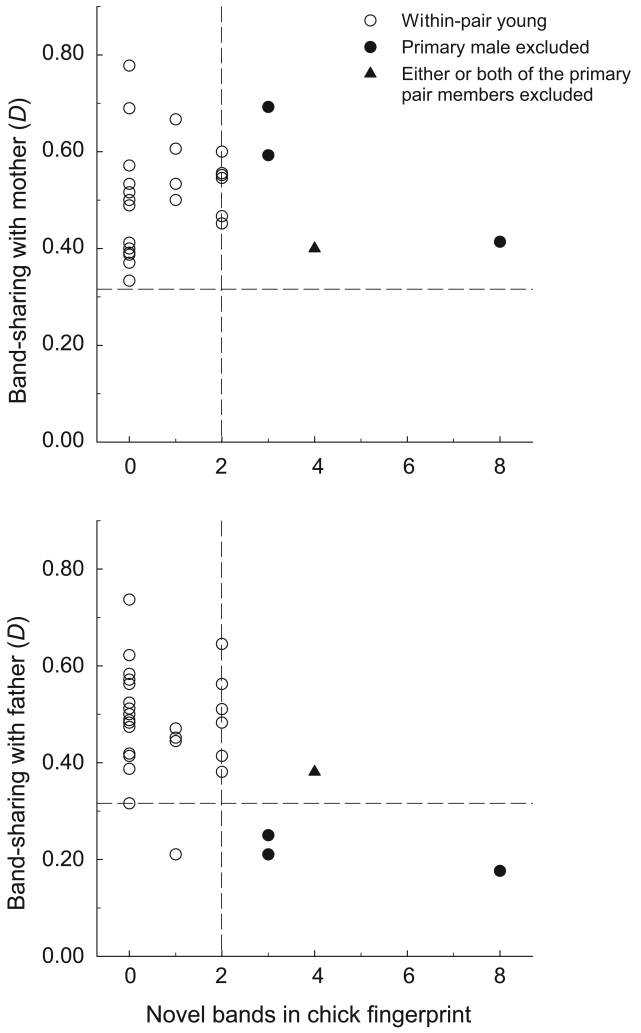

FIGURE 1. Results of DNA fingerprinting of Southern Lapwings breeding in Brasilia, Brazil, 2000–2002. Data from nine breeding pairs or groups for which blood samples were obtained from both of the primary pair members (presumed parents) and their chicks ($n = 29$ chicks from 11 broods). The figure shows the association between number of novel bands in chick fingerprints and band sharing with the primary pair female (top) and male (bottom). The band-sharing coefficient D denotes the proportion of DNA fragments shared between two individuals, calculated as described by Wetton et al. (1987). The horizontal dashed line indicates the lowest expected band sharing between genetic parents and their young, and the vertical dashed line, the highest number of novel bands expected due to mutation or other random causes.

observed any copulations between the male or female of a primary pair with one of the additional adults in the group. All genetic mismatches between primary pair members and offspring were recorded in breeding groups (3–4 adults). However, the occurrence of such mismatches did not differ significantly between groups (3 of 10) and secluded pairs (0 of 10; two-tailed, Fisher exact test, $P = 0.23$). Including multiple breeding attempts by the same primary pair and using broods as the sample unit yielded a similar result ($P = 0.22$).

DISCUSSION

We have shown that breeding Southern Lapwings have a variable social structure as well as a flexible genetic mating system. Ten territories were occupied by socially and genetically monogamous pairs, while a second social structure involved ten groups of either three or four adults. In three of these groups, we found genetic mismatches between chicks and their presumed fathers, thus representing genetic polygamy. The frequency of mixed paternity in Southern Lapwings (18.8% of broods, 9.8% of chicks) is higher than the rates typically found in socially monogamous shorebirds, but similar to some socially polyandrous species (Küpper et al. 2004). We recorded mixed paternity only in breeding groups, which seems consistent with results from the Wattled Jacana (*Jacana jacana*), a socially polyandrous shorebird. In that species, Emlen et al. (1998) recorded no paternity loss when a female was paired with only one male, whereas such loss was frequent in polyandrous groups. Further work is required to determine whether there is a difference in the mixed paternity rate between pairs and breeding groups in Southern Lapwings, and whether multiple paternity in groups arises through shared reproduction or through copulation with individuals outside the group.

An interesting finding was that one chick shared a high proportion of bands with both members of the primary pair of its unit despite having four novel bands in its genetic profile. The low probability of mutations resulting in four novel bands led us to classify the chick as a genetic mismatch with either or both of the parents. However, it was likely a mismatch only with the father since we found no additional genetic evidence of female brood parasitism. Also, there were no obvious differences among eggs, either in pigmentation or size (visually estimated), that would have indicated that more than one female was laying eggs in nests of either pairs or groups. Thus, the most plausible explanation for this case is that the female copulated with a relative of her social mate. However, we cannot rule out quasi parasitism. In such a case, the chick would have the same father as its nest mates but a different genetic mother, probably a relative of the tending female. Quasi parasitism has been recorded in a related species, the Kentish Plover (*Charadrius alexandrinus*; Küpper et al. 2004).

Mate guarding has been defined as any behavior by a paired male that decreases the chances of his female copulating with another male during her fertile period (Birkhead 1979, Møller and Birkhead 1991). Both male following behavior and frequent copulation can be tactics to assure paternity (Blomqvist et al. 2002). Additionally, frequent copulation, such as reported in Northern Lapwings (*V. vanellus*), has been interpreted as a mechanism to promote pair bonding and communication between pair members, leading to increased breeding success (Zöllner 2001). During our frequent, though brief visits to territories, we observed several copulations by the primary pair members, either as secluded pairs or in groups. It is possible that copulation in Southern Lapwings may have a similar function as those described above, since we never observed copulations between primary pair members and the additional adults in groups (in which all cases of mixed paternity were found). However, further data are needed before a functional significance can be assigned to the copulatory behavior observed. Moreover, it is possible that copulation between primary pair members and extra adults was not detected because it may have occurred at night, a common pattern in some temperate shorebirds (Johnson et al. 2002).

To summarize, our study has revealed variable reproductive behaviors in Southern Lapwings. We found that the social structure

includes pairs of monogamous birds, but breeding groups are also common and may be a reproductive alternative for the species. However, many intriguing questions regarding the Southern Lapwing's social and genetic breeding system remain. As we were unable to capture the additional adults in breeding groups, it is possible that such groups can exhibit a classic helpers-at-the-nest structure, consisting of a single family. Additionally, we could not determine the sex of the extra adults, which may influence the establishment of different mating patterns and raise questions about how the sex composition of groups affects mate guarding and competition for fertilizations.

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