

Testosterone-induced male traits in female ruffs (*Philomachus pugnax*): autosomal inheritance and gender differentiation

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A balanced polymorphism in male mating behaviour exists in male ruffs, with no obvious parallel expression in females. Pedigree data of male phenotypes support an autosomal model of inheritance, in contrast to sex-linked inheritance patterns found in other taxa with sex-limited alternative mating strategy polymorphisms. We tested this model by inducing male courtship behaviour in gonad-intact female ruffs, using subcutaneous testosterone implants that produced physiological concentrations of testosterone. The implants rapidly induced in females both types of male mating behaviour, an increase in body mass typical of pre-breeding males, and the growth of normally male-limited breeding plumage. As predicted under an autosomal model, the distributions of induced male behaviour types in females paralleled those of their brothers and half-brothers, and were inconsistent with sex-linked models. Effects were reversible, and experimental females bred normally in subsequent years. Our results show that genotype-specific male characteristics can be induced by testosterone in female adults that have presumably not undergone neural organization for them early in life, showing direct use of genetic information in intra- and intersexual differentiation.

Keywords: activation–organization; alternative strategies; genetic models; hormones; polymorphism; sexual differentiation

1. INTRODUCTION

Males and females share their DNA, except for genes located on heteromorphic regions of sex-determining chromosomes. What largely differentiates the sexes, physically, physiologically, and behaviourally, are the patterns of gene activation throughout ontogeny. When selection favours different traits in males and females, organisms commonly alter the expression of autosomal loci conditional on sexual identity, both during critical periods early in development and later in life (Adkins-Regan 1987; Etches & Kagami 1997). For many taxa, an additional mechanism is to use loci located on, or to evolve, heteromorphic sex chromosomes. Loci expressed exclusively in a heterogametic sex will be less subject to antagonistic pleiotropy between the sexes (e.g. Lande 1980, 1987; Rice 1984; Hastings 1994; Wilcockson *et al.* 1995), and selection will proceed more rapidly for sex-linked characters, since recessive alleles are more frequently exposed to selection (Rice 1984; Charlesworth *et al.* 1987). Coding for sex-limited traits should thus be found disproportionately on sex chromosomes. Empirically, X-chromosomal loci in insects and mammals affect sexually selected traits disproportionately strongly relative to non-sexually selected traits (Reinhold 1998), and mammalian Y chromosomes code predominantly for characteristics directly related to sperm production and sexual differentiation (Lahn & Page 1997; Roldan & Gomendio 1999).

Alternative reproductive behaviours within sexes are characteristics on which sexual selection has the obvious potential to operate strongly, and thus sex-linkage may be particularly likely for such traits. In nearly all taxa where alternative reproductive behaviours are found, the use of one behaviour or another reflects phenotypic plasticity, and thus genetic variation for behavioural alternative *per se* is unimportant (Eberhard 1982; Emlen 1994; Gross 1996). However, in a small number of species, individuals do carry alternative alleles for reproductive strategies. In some, both sexes express polymorphic characteristics (seaweed fly, *Coelopa frigida* (Day & Gilburn 1997); white-throated sparrows, *Zonotrichia albicollis* (Tuttle 1993); side-blotched lizards, *Uta stansburiana* (Sinervo & Lively 1996)), while others have sex-limited polymorphisms (marine isopod, *Paracerceis sculpta* (Shuster & Sassaman 1997); species of poeciliid fish (Kallman 1983; Borowsky 1987; Ryan & Wagner 1987; Zimmerer & Kallman 1989; Erbelding-Denk *et al.* 1994)). In the species listed above with polymorphisms in both sexes, autosomal models of inheritance have been proposed. In contrast, inheritance patterns that involve sex-linked loci best fit the data for the species with sex-limited characteristics.

Lank *et al.* (1995) rejected sex-linked (Z-linked in birds) models for the inheritance of a well-described dimorphism in male mating behaviour in the ruff *Philomachus pugnax* (Hogan-Warburg 1966; Van Rhijn 1991; Hugie & Lank 1997; Widemo 1998), and supported an autosomal single-locus, two-allele model with complete dominance. The ruff is a lekking sandpiper that breeds

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from northern Europe through Siberia. Throughout this range, there are two male behavioural morphs. 'Independent' male ruffs establish lek courtship territories, which they defend against other independents. 'Satellite' males, representing around 16% of populations, share courts with independents in uneasy transient alliances that appear to be stabilized by female preferences for choosing mates when both morphs are present (Hugie & Lank 1997; Widemo 1998). Independent and satellite males also differ in body size and patterns of body mass variation during the breeding season (Bachman & Widemo 1999), and in the coloration of their highly polymorphic breeding plumages, particularly the long fluffy display plumes that ring their neck (the 'ruff') and occipital 'head tufts' (Hogan-Warburg 1966).

Female ruffs, termed 'reeves', have no obvious behavioural expression of the variation seen in males. Knowing the distribution of genotypes carried by reeves, the heterogametic sex, would further discriminate between autosomal and sex-linked inheritance. Sex-linked models predict that phenotype frequencies in the heterogametic sex represent allelic frequencies, since only one allele is present, rather than representing genotype frequencies, as predicted for autosomal inheritance. Exogenous testosterone can induce components of male behaviour and morphological characteristics in adult females of many species of birds (Emlen & Lorenz 1942; Witschi 1961). Castrated male ruffs fail to moult into breeding plumage (Van Oordt & Junge 1936), suggesting that testosterone is essential for this process. We therefore administered testosterone to pedigreed reeves in an attempt to elicit a phenotypic expression of their genetic variability for male-limited traits, and to test models of inheritance of this character.

2. METHODS

(a) *Subjects and pedigrees*

A captive breeding flock of ruffs has been maintained in Canada, established from eggs collected near Oulu, Finland, in 1985, 1989 and 1990. We implanted 112 reeves from this flock with testosterone immediately following breeding seasons in 1991–1995 ($n=10, 33, 32, 21, 31$ per season, respectively; 15 reeves were implanted in two different years). The paternity of 91 of these reeves was known because their mothers had bred in captivity while segregated with a single male ($n=82$), or was determined by minisatellite DNA fingerprinting of eggs collected in the wild ($n=9$; Lank *et al.* 1995). Twenty-one implanted reeves, also raised from eggs collected in the wild, had unknown fathers. These eggs were collected from a population with 84% independent males, thus we expect a strong bias towards independent paternity.

(b) *Testosterone implantation*

Testosterone (Sigma Chemical Co., St. Louis, MO, USA) implants (silastic tubing inner diameter 0.147 cm, outer 0.196 cm; Dow Corning, Mississauga, Ontario, Canada), sealed with silastic glue, produced *ca.* 1.84–2.46 cm² of implant surface area. Implants were inserted subcutaneously through a 1 cm incision in an apterium along a bird's flank, which was closed with a suture or cyanoacrylate glue (Vetbond[®]). Implant position was checked periodically, and a reeve was reimplanted on the opposite side if the implant became dislodged or exposed to the air.

Each season, reeves were implanted on one or two adjacent dates between 1 and 5 July. Implants were removed between 38 and 70 days later, at which time we scored the presence and colour of male display plumage feathers the females had grown.

To determine whether circulating levels of testosterone were comparable with those of breeding males, we collected blood samples 21 days after implantation from the ten reeves implanted in 1991. Blood was drawn from the brachial vein into capillary tubes (75 µl) or Hamilton syringes, immediately separated and the serum was stored at -20°C until assayed for testosterone content. An ¹²⁵I total testosterone radioimmunoassay kit (Diagnostic Products Corporation, Inter-Medico, Markham, Ontario, Canada) was used to quantify duplicate 50 µl samples, with a lower limit of sensitivity of 0.8 ng ml⁻¹ of serum. We report mean determinations for each reeve.

(c) *Behaviour quantification*

Implanted reeves were housed in groups of 10–20 under natural photoperiods in outdoor pens located near Kingston, Ontario (1991–1993) and Burnaby, British Columbia (1994–1995). In 1991 only, eight control, unimplanted reeves were housed with the implanted flock. Behavioural observations of the 'male' courtship and social behaviour induced in these implanted reeves were taken in July and August by students familiar with male display from having watched captive breeding ruffs the previous spring. Observers had no knowledge of reeve pedigrees.

(i) *Reference data for male phenotype*

Although displaying adult males are readily classified as independents or satellites (Hogan-Warburg 1966, table 10; Van Rhijn 1973), we generated a quantitative description of independent and satellite behaviour under captive conditions against which implanted reeve social behaviour could be scaled. We sampled two groups of eight independent and two satellite males for 3 min per male during each of 12 observation periods between 15 and 26 May 1996. Two unimplanted reeves were present to stimulate male display. We scored 20 courtship displays and social behaviour patterns, briefly described in table 1. We tallied particular displays or social behaviour patterns only once within 30 s intervals. Social interactions near food bowls or water baths were ignored. We summarized morph behaviour by calculating the mean frequencies and probability of occurrence of each behaviour in independents versus satellites, using each bird as a single data point (table 1).

(ii) *Behaviour of implanted females*

We summarized each implanted reeve's behaviour as a single 'male phenotype index' score (MPI), ranging from 0, indicating exclusively satellite behaviour, to 1, indicating exclusively independent behaviour. To provide comparable variance structures for data collected differently among seasons, we defined a bird's MPI as a mean of means from sampling sessions.

The 1991 data consisted of 74 one-minute samples taken on days throughout the implant period, with behaviour tabulated as done for the male reference data. The 1995 data consisted of 37 consecutive daily samples starting two days after implantation. All reeves were sampled for 1 min each day, and a subset of actively displaying reeves was selected daily and scored for an additional 4 min each. We pooled data into 12 three-day sampling sessions to dampen variance generated by 1 min samples. For each sampling session in 1991 and 1995, we

Table 1. Names, descriptions, and references for displays and social behaviour scored for males and implanted females, and rates and relative probability of their use by captive independent and satellite males

(Behaviour patterns at the top were considered qualitatively diagnostic of independents, and those at the bottom diagnostic of satellites. In nature, all courtship displays listed, except 'rouch when courted', are exclusively seen in males. The social behaviours chase, fight, and peck occur in females, but not in the context of courtship behaviour. This table does not fully represent the behaviour probabilities of males in the wild. $n = 16$ independents, four satellites. HW66, Hogan-Warburg (1966); VR 73,91, Van Rhijn (1973, 1991).)

courtship display or social behaviour	description or reference	figure number in			mean min ⁻¹		relative probability of occurrence by independents
		HW66	VR73	VR91	independents	satellites	
independent							
forward	head lowered, body horizontal, bill pointed towards another bird	3, 9	1 <i>h</i>	—	0.247	0.000	1.0
forward with bill thrust	forward with head jerks thrusting bill towards opponent	4	—	—	0.224	0.000	1.0
bill point other bird	upright male points bill at another's head; male or female recipient often crouches in response, not described from nature	—	—	—	0.048	0.000	1.0
chase other bird	run towards other bird, often in forward posture	—	—	—	0.046	0.000	1.0
fight other bird	mutual agonistic behaviour, with contact	—	—	p. 8	0.036	0.000	1.0
mutual squat, top	independent partially squatted or forward with bill on or near head of co-occupying satellite, see also Hugie & Lank (1997, fig. 1 <i>c</i>)	16	—	17	0.026	0.000	1.0
peck other bird	hit bird with bill, pecked bird does not reciprocate	—	—	—	0.025	0.000	1.0
crane neck other bird	upright male approaches male or female with bill pointing down; recipient often crouches in response, not described from nature	—	—	—	0.025	0.000	1.0
pecked by other	be hit by other bird without reciprocal threat	—	—	—	0.011	0.000	1.0
mutual squat, circle	as mutual squat, top; independent circles or half circles satellite	diag. 2	—	—	0.018	0.000	1.0
probabilistic							
half squat	body horizontal, legs bent, ruff erect, bill down	—	1 <i>e</i>	16	0.346	0.032	0.915
full squat	'squat' in literature, as half squat with tarsi on ground	16	1 <i>f</i>	16	0.229	0.038	0.856
chased by other	be chased by other bird, without reciprocation	—	—	—	0.029	0.008	0.774
oblique, solitary	body 45° angle, ruff feathers erect, no bird within 0.2 m	2, 10	1 <i>c</i>	—	0.539	0.576	0.481
satellite							
receive bill point or crane neck	receive attention from bill pointing or crane-necking individual	—	—	—	0.002	0.242	0.007
upright on other's court	upright within 0.2 m of bird behaving as independent	13	—	16	0.000	0.742	0.0
oblique on other's court	oblique within 0.2 m of a bird behaving as an independent	—	—	16	0.000	0.511	0.0
mutual squat, bottom	satellite full squatted with independent mutually squatted on top see also Hugie & Lank (1997, fig. 1 <i>c</i>)	16	—	17	0.000	0.096	0.0
crouch when courted	crouch or squat, apparently in response to male attention; satellites and females show this behaviour	—	1 <i>f,g</i>	17	0.000	0.019	0.0
upright, solitary	body vertical, neck stretched upwards, neck ruff partially erect	5, 6	1 <i>a</i>	—	0.000	0.006	0.0

Table 2. *Behavioural morph of brothers and paternal half-brothers of implanted reeves, categorized by the morph paternity of the father; and the proportion of testosterone-implanted females expected to behave as independents under autosomal and sex-linked genetic models*

(The autosomal prediction derives from genotype proportions, which have identical expectations in males and females. The predictions for the sex-linked models, where S,s , satellite allele dominant and I,i , independent allele dominant, are the allelic frequencies estimated for males under each model.)

paternal morph	number of sons		proportion of testosterone-implanted females expected to behave as independents		
	independents	satellites	autosomal model	sex-linked models	
				S,s	I,i
independent	45	2	0.96	0.98	0.68
unknown	13	2	0.87	0.93	0.62
satellite	20	22	0.48	0.73	0.68

computed mean MPI scores by multiplying frequencies of behaviour by the probability of its being shown by an independent (table 1), and then averaged the seven or 12 means for each reeve. These procedures produced MPI values nearly identical to those calculated by simply pooling all observations per bird (1991 and 1995 data: Pearson's $r = 0.94$, $n = 41$, $p < 0.0001$).

In 1992–1994, implanted reeve behaviour was recorded *ad libitum* for periods of 1 to 3 h on four, seven and four days per year, respectively. Observers focused their attention on actively displaying reeves, but ensured that every reeve was identified during each observation period. If all behaviour recorded was diagnostic and consistent with identity as an independent or satellite (table 1), the data were summarized with a single entry per session. If probabilistic behaviour patterns were recorded, or if both diagnostic independent and satellite behaviour patterns were recorded (one case), we included multiple entries per day. MPI scores were calculated as the means of all entries per reeve. This procedure is conservative with respect to dichotomizing reeve behaviour because observers were biased against recording repeated records of consistent behaviours, and inconsistent behaviour is overrepresented. The distributions of MPI scores with respect to paternal phenotypes did not differ among seasons (G -tests from multiway contingency table analysis, using five equally spaced MPI bins, $p > 0.10$ for all interactions with season).

For the five reeves with MPI values from two seasons, we used the value from the season for which we had more data. None showed inconsistent behaviour. Four reeves failed to show male display behaviour, and were excluded from the analysis.

(d) Genetic models

The autosomal genetic model (Lank *et al.* 1995) states that a satellite allele (S) is dominant to an independent allele (s), but present at low frequencies ($S = 0.08$, $s = 0.92$) and that nine out of ten satellites are heterozygotes in natural populations. To generate phenotypic expectations for the implanted reeves, we compared the distributions of male behavioural phenotypes induced in implanted reeves with the distributions of phenotypes expressed by their full and paternal half-brothers. To obtain statistical power, we pooled offspring by paternal category (independent, satellite or unknown father). Under an autosomal model, the distribution of genotypes has the same expectation for both sexes, and brothers and sisters should have similar phenotype distributions. Under a sex-linked (Z-linked) model, the distribution of behaviour of the heterogametic sex should match the allelic frequencies instead, and we would thus expect

to see a higher proportion of recessive characteristics expressed in implanted reeves than in their brothers. For each pedigree group, we calculated allelic frequencies of sons under a satellite allelic dominance (S,s), or an independent allelic dominance (I,i) Z-linked model, using population genetic assumptions about allelic frequencies from table 2 in Lank *et al.* (1995). Under Hardy–Weinberg equilibrium, the satellite dominance model predicts that 94% of satellites are Ss heterozygotes, and the independent dominance model predicts that 57% of independents are Ii heterozygotes. We used these values to calculate putative allelic and genotype frequencies under each genetic model for each of the three paternity groups of brothers, and calculated the proportion of implanted reeves expected to behave as independents (table 2).

3. RESULTS

(a) Effects of hormone implants

The ten reeves sampled in 1991 had circulating levels of testosterone distributed as follows: two below the sensitivity of the assay (0.8 ng ml^{-1}), one at 1 ng ml^{-1} , six at 2 ng ml^{-1} and one at 3 ng ml^{-1} . Both birds with undetectable levels of testosterone at the time sampled had shown behavioural and plumage responses. These levels are similar to those seen in breeding males (unpublished control pools measured with the same assay), and thus the implants produced physiological concentrations of testosterone.

Within 48 h after implantation, reeves began performing typical male epigamic displays (table 1). Within a week, groups of implanted reeves had established a 'lek' on which many females defended *ca.* 1 m^2 territories comparable with those of independent males. None of the control reeves observed in 1991, nor other unimplanted females we have watched in captivity, showed these courtship displays or social behaviour patterns.

Implanted reeves gained more mass during the subsequent two months than did control reeves (1991 data: at implant, controls = $93.6 \pm 2.1 \text{ g}$, implants = $92.5 \pm 1.4 \text{ g}$, $n = 8, 10$, n.s.; on day 20, controls = 94.7 ± 2.6 , implants = 106.0 ± 3.1 ; on day 55, controls = 101.3 ± 7 , implants = 116.1 ± 4.7 ; repeated-measures ANOVA, mass \times group effect, $f = 7.4$, 2 d.f., $p = 0.002$).

After three to five weeks, 106 of the 112 implanted reeves had grown a neck ruff of long filamentous male-like display feathers, distinct in structure and colour from

normal contour feathers. As in males, these feathers varied in pigmentation and colour pattern. Reeves grew black, rusty, or creamy feathers, many with darker flecks, tips, or barring, which fall within the spectrum of normal male display feathers on the neck ruffs of males. However, no reeve grew pure white feathers, as do some males. Although 95% of females grew distinctive ruff feathers, only three also grew discernible head tuft feathers. Four females also developed small numbers (< 20) of the temporary facial 'wattles' present in adult males during the breeding season.

(b) Male reference data

Independent and satellite males showed clear differences in frequencies and probabilities of behaviour patterns (table 1). Since 'solitary obliques' provided no discrimination of male phenotypes, we excluded them when calculating MPI scores for reeves.

The phenotype distribution of the brothers and half-brothers of the implanted reeves was as expected under the autosomal genetic model proposed previously (Lank *et al.* 1995, table 2), which was derived in large part from a subset of these data. The parental females of these families were predominantly hypothesized homozygous recessives, *ca.* 84% in the parental population. Independent fathers, hypothesized homozygous recessives, produced 96% independent sons. Satellite fathers, nearly all presumed heterozygotes, produced an even ratio of independent and satellite sons. Males of unknown paternity, all raised from eggs collected in the wild, produced 87% independent sons, as close as possible to the frequency expected from a random sample of this population.

(c) Behaviour of reeves with different pedigrees

Daughters of independents and satellites had different distributions of MPI scores (figure 1). Independent daughters ($n=54$) had a unimodal distribution strongly peaked at 1, indicating independent behaviour, but two daughters had extremely low scores, indicating satellite behaviour. Satellite daughters ($n=33$) had a flat, bi- or trimodal distribution, significantly different from that of independent daughters (with five bins of 0.2 each: $n=87$, 4 d.f., $G=20.2$, $p > 0.001$). Distributions from daughters of unknown fathers ($n=21$) matched those of independents ($n=75$, $G=1.4$, 3 d.f. (no data in bin 0.02–0.04), n.s.), as expected from a sample of a population with *ca.* 84% independent parents.

There is strong agreement between the morph distributions of sons and the MPI distributions of implanted daughters, for all three paternity classes (c.f. figure 1 versus table 2). Both male and female offspring of independents, and those with unknown, but probable independent paternity, are nearly all independents. In contrast, male offspring from satellites are split evenly between morphs, and females spread across the range of MPI values. Both outcomes are as predicted by the autosomal model.

We cannot test the expected proportions generated by the three genetic models presented in table 2 against the observed female data because there is no reasonable way of dichotomizing reeves as independents versus satellites. We attempted to do so using non-parametric discriminant function analysis, but this failed to provide meaningful assignments because the male reference data set is too

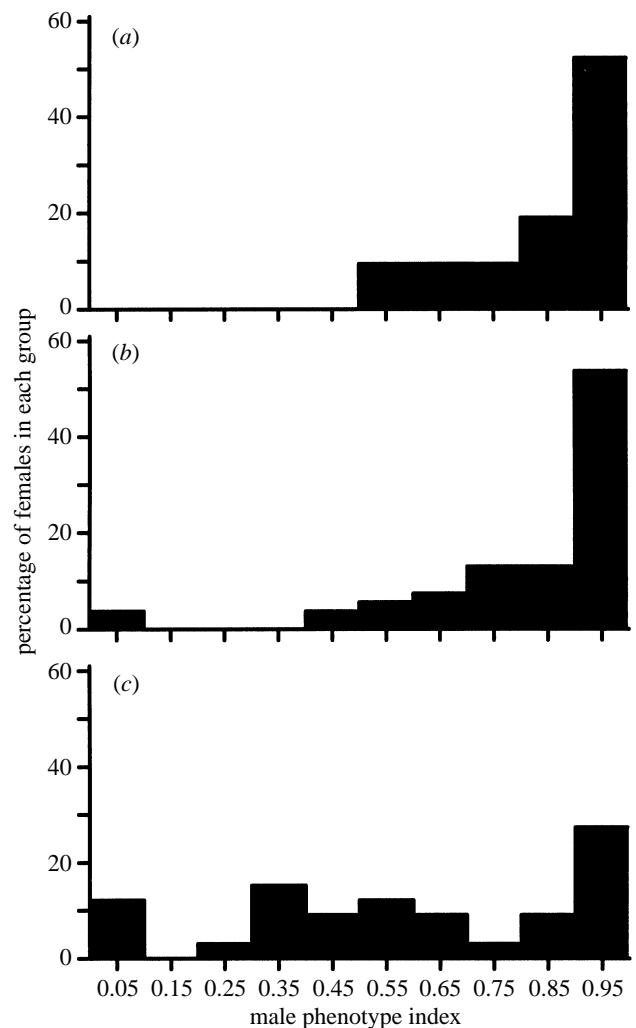


Figure 1. Distribution of MPIs among testosterone-implanted females differing in paternal morph. (a) Father unknown, collected in the wild, $n=21$; (b) independent father, $n=54$; (c) satellite father, $n=33$.

dichotomous to provide reliable assignment probabilities for intermediate cases. However, both Z-linked models make predictions that are inconsistent with the data. The Z-linked independent allelic dominance model (I_i) predicts similar distributions among all three offspring classes (*ca.* 0.62–0.68 independents), which was not observed, since the distributions differ (shown above). For the Z-linked satellite dominance model (S_s), an independent proportion of 0.73 for satellite offspring would occur if we dichotomized females at a MPI value of around 0.30. However, the unimodal distributions of daughters of independents and unknowns suggest a cut-off value would be no lower than the 0.45 bin.

4. DISCUSSION

(a) Responses to testosterone implantation

Testosterone implants in gonadally intact reeves induced male courtship behaviour within days, caused an increase in reeve body mass over several weeks, and stimulated the growth of male display feathers. We noted no alterations in the post-experimental behaviour of implanted reeves, which reproduced successfully in subsequent years.

At the start of the breeding season, breeding males, and independents in particular, have body masses that are 15–30% higher (20–60 g) than during the winter (Scheuffler & Stiefel 1985). Most of this increase is fat, which is slowly lost by independents because time committed to maintaining lek courts precludes feeding (Lank & Smith 1987; Bachman & Widemo 1999). The approximately 15% increase in mass by implanted reeves paralleled this pre-breeding physiology.

Testosterone induced the development of male display feathers, as previously suggested by the lack of growth of such feathers in castrated males (Van Oordt & Junge 1936). Our dramatic confirmation of this is of interest in part because elevated testosterone levels are generally not involved in the production of male breeding plumage in birds (Owens & Short 1995), although they may be more important for Charadriiformes than other groups (Kimball & Ligon 1999). Alternatively, the display feather moult of ruffs is considered a novel 'supplemental' addition to the two feather generations grown per year by most shorebirds (Jukema & Piersma 2000), and the mechanisms controlling this derived character may differ accordingly.

No females grew the pure white display feathers typical of ca. 20% of males, including most satellites. Display feather pigmentation may have interacted with the grey-brown pigmentation of a female's normal contour feathers. Alternatively, some display plumage coloration modifiers may be sex linked, as in chickens (Smyth 1990), and thus produce different outcomes when expressed in the two sexes.

To our surprise, only four implanted birds grew discernible head tufts. The normal post-breeding moult occurring in females in early July starts with the head. Thus, few head tufts may have grown if head feather structure had been established prior to implantation. Alternatively, the mechanism regulating head tuft growth may differ from that of the ruff, quantitatively or qualitatively. If so, these two plumage characters could signal different aspects of a male's previous physiological condition, or 'quality', that females or other males might assess, as predicted by theories addressing the evolution of multiple male ornaments (Møller & Pomiankowski 1993).

Administration of testosterone to elicit expression of normally sex-limited characters has only rarely been exploited to ask questions related to genetics, mate choice, or the behavioural basis of population structure (e.g. guppies, *Lebistes reticulatus* (Haskins *et al.* 1961); passerine birds (Baptista & Morton 1982, 1988)), but the technique has tremendous potential in these areas.

(b) Genetics of sexually dimorphic and sexually selected traits

Our results support the existence of a single locus autosomal genetic mechanism for the inheritance of alternative male phenotype in ruffs. Testosterone induces meaningful male behavioural phenotypes in reeves, and the distribution of these phenotypes parallels that of their brothers, as this model predicts. One purely practical outcome of this is that future analyses can consider offspring ratios produced by crosses with known phenotypes of both parents, rather than testing models that rely

on male phenotypes only and population genetic assumptions about the genotypes of females (Lank *et al.* 1995).

The genetic architecture of this 'single locus' is unknown. Avian genomes are packaged in a small number of macrochromosomes (e.g. four to six) plus numerous microchromosomes. In white-throated sparrows, a plumage and behavioural polymorphism expressed in both sexes correlates perfectly with a karyotypically visible chromosomal inversion on one of the macrochromosomes (Thornycroft 1975), producing a 'single locus' inheritance pattern for these traits that subsumes differentiation at multiple loci within the linkage group (e.g. Day & Gilburn 1997). A similar inversion for behavioural phenotype may exist in ruffs, allowing the accumulation of correlated alternative alleles persisting in linkage disequilibrium. A process of 'morph chromosome evolution', somewhat parallel to the evolution of sex chromosomes (Bull 1983; Charlesworth 1991), might be occurring in both of these taxa. Alternatively, the 'single locus' could be exactly that, with male phenotype differentiating traits cascading from differences triggered by a single alternative set of alleles.

The conditions for maintaining polymorphisms are more restrictive for sex linked than for autosomal traits (Hendrick & Parker 1997). Nonetheless, polymorphisms for intrasexual variation in mating strategy appear to exist in isopods and poeciliid fish (see §1). Sexual selection may have acted unusually strongly in these cases. Does a general pattern of sex linkage for alternative male reproductive traits exist? While the isopod example represents an independent evolutionary event, the poeciliid fish examples could reflect a single ancestral event. However, variation among the genetic models proposed (see references in §1) suggests multiple origins, or at least continuing evolution, within in this clade. This tentative pattern may genuinely reflect selection favouring sex-linked genetic mechanisms for such traits.

Ruffs have an autosomal genetic mechanism perhaps simply because of the ZW sex determination genetics of birds. Since males are the homogametic sex (ZZ), a trait affecting males on the Z will evolve no more rapidly than autosomal traits. A parallel case may occur in dragonflies and relatives, where numerous species have genetically based colour and behaviour polymorphisms that are sex-limited to females, and apparently controlled by autosomal loci (Johnson 1966; Cordero 1989). Since females are the 'homogametic' sex in the odonate XO system, there are no female-limited loci to carry such traits. Male dimorphism in colour and behaviour that may also reflect genetic polymorphisms occurs in certain odonate genera (e.g. Tsubaki *et al.* 1997), but unless these genera deviate from XO sex determination, there would also be no male-limited loci on which the polymorphism might occur.

(c) Inter- and intrasexual gender differentiation

The dramatic responses of reeves to testosterone implantation place courtship behaviour, mass gain, and display plumage growth in ruffs alongside other gender-specific traits that can be induced by steroids in adult vertebrates in the absence of 'organization' of neural or other structures early in life. For birds, a 60 year literature shows that steroids can readily induce varied components of transsexual behaviour, and morphological

changes such as wattle growth in chickens (*Gallus gallus*), both in intact and castrated adults (e.g. Crew 1923; Emlen & Lorenz 1942; Witschi 1961, Adkins & Adler 1972; Baptista & Morton 1982, 1988; Etches & Kagami 1997). These traits contrast with those for which organization by steroid hormones produced by developing gonads during sensitive periods of embryonic development produce permanently gender differentiated brain and other secondary sexual structures, which may subsequently 'activated' by steroid regimes later in life (e.g. puberty, seasonal breeding) (Adkins-Regan 1987; Etches & Kagami 1997). Accounting for our results with this model requires that females had previously developed, for other reasons, specialized structures to facilitate the responses. Although females must carry neural 'templates' to recognize male characteristics used in mate choice, it is doubtful that these neurological structures influence the neuromuscular centres controlling the performance of displays (e.g. Brenowitz *et al.* 1994), let alone the mechanisms responsible for feather development, where sexually differentiated distributions of local steroid receptors have been identified in domestic geese, *Anser anser* (Péczy 1992).

Moore (1991) and Hews *et al.* (1994) proposed that the organization-activation model might be generalized to account for alternative breeding tactics or strategies within sexes, as well as differentiating between them. Applied to ruffs, this hypothesis predicts that (i) differential circulating levels of steroids within males during neonatal development might organize independent versus satellite neural or other structures, and (ii) later in life, morph-specific differences in behaviour or morphology could occur despite similar hormonal regimes. This latter prediction may hold, since implantation of testosterone into adult males did not change their behavioural morphs (D. B. Lank and K. E. Wynne-Edwards, unpublished data). However, for the model to account for our major results, reeves would have had to previously undergone intrasexual differentiation, implying that dimorphism for breeding behaviour occurs in both sexes. A failure to recognize female dimorphism would not in fact be surprising, since no studies of individual female mating behaviour have been published, and the pedigrees of wild reeves are unknown. Breeding reeves do vary in the coloration of the upper breast, and this variation correlates with their paternal pedigree and the predominant colour of their testosterone-induced male display feathers (D. B. Lank, unpublished data). However, differently coloured reeves do not obviously behave differently in female-female or male-female social interactions or mating behaviour. The differentiated responses of females by pedigree suggest direct usage of variable genetic information by implanted females, and it is reasonable to assume that a similar mechanism also normally operates for these characters in males. Our results thus do not support a major role for organizational effects for important aspects of sexual or male morph differentiation of ruffs.

(d) Environmental implications

The extraordinary ease with which sex-limited traits were elicited from reeves has cautionary environmental implications. In male mammals, where gestation is

internal and foetal-maternal contact is intimate, phenotypic sex determination includes critical period masculinization' during embryonic development, involving deactivation of responsiveness to inappropriate-sex endocrine pathways and activation of appropriate-sex pathways. The value of protecting the foetus against potential interference from maternal circulation could explain why organizational effects appear to be stronger in mammals than in other vertebrates. As an incidental side-effect, these processes may partially protect mammals from disruptive effects of endocrine-mimicking chemicals. In birds, fishes, and other organisms in which embryonic development is external and self-contained, natural selection may not have deactivated inappropriate pathways because there is no equivalent risk of inappropriate endocrine signalling via the maternal circulation. Egg-laying species maybe particularly sensitive to exogenous, inappropriate sex steroids, or their mimics, that can transform their development, morphology or behaviour.

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