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Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*)

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Abstract The evolution of multiple sexual traits remains controversial and poorly understood in evolutionary biology. In many bird species, males exhibit complex courtships involving feather ornaments and behavioral display. Multiple traits may convey information on the genetic and phenotypic quality of males. In particular, fixed characters, such as feather ornaments (produced once a year during the annual molt in many bird species) might convey information about past male condition (at the time of trait development); whereas flexible traits such as behavioral displays should be sensitive to present condition. Here we show that both flexible behavioral displays and fixed feather ornaments of peacocks, used by females to choose a mate, honestly reflect health status. Correlative data showed that peacocks with high display rate (the number of behavioral displays per hour) and a large number of tail eyespots had low levels of circulating heterophils, suggesting better health status. Experimental activation of the immune system, through LPS injection, significantly reduced display rate compared to a control group. However, the sensitivity of a male display rate to the immune challenge

was dependent on the number of tail eyespots: males with higher number of tail eyespots were better able to cope with the experimental immune challenge, and maintained their display rate at levels closer to the levels performed before the immune activation. These results are consistent with the idea that multiple signaling might enhance information reliability.

Keywords Female choice · Immune challenge · Multiple traits · Sexual selection

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Introduction

Male sexual advertisements affecting either male–male competition or female choice often involve several morphological and behavioral traits (Andersson 1994). Much emphasis has been put on the role played by female choice in the evolution of multiple male traits. Verbal and mathematical models have been used to explore the different scenarios under which multiple male traits are selected and maintained by female mating preference (Møller and Pomiankowski 1993; Pomiankowski and Iwasa 1993; Iwasa and Pomiankowski 1994; Johnstone 1995, 1996). Whereas some models have suggested that multiple male traits are unlikely to be “handicap revealing” (Zahavi 1975; Grafen 1990) – unless the cost of mate choice is low (Iwasa and Pomiankowski 1994) – others have shown that good gene selection can drive the evolution of multiple male traits (Johnstone 1995). Empirical tests of these predictions are still rare. Recently, Andersson et al. (2002) studied the function of multiple signals in red-collared widowbirds (*Euplectes ardens*) and found that tail length and the red carotenoid collar badge were involved in inter- and intra-sexual selection respectively. Interestingly, the two traits were negatively correlated and this trade-off might explain the coexistence of multiple honest signals. In other species, multiple male traits serve only in the process of female choice. In this case multiple displays could signal different properties of male condition (multiple message hypothesis)

or they could reinforce the reliability of female choice (Sullivan 1994; Rowe 1999). For instance, female satin bowerbirds (*Ptilonorhynchus violaceus*) assess male plumage UV coloration as well as bower features to choose mates. Bower and plumage indicate different aspects of health, the ectoparasite load and the intensity of infection from blood parasites respectively (Doucet and Montgomerie 2003).

The peacock has long been considered as a prime example of the strength of sexual selection on the evolution of multiple sexual traits (Darwin 1871; Petrie et al. 1991, 1992; Petrie 1994; Petrie and Halliday 1994; Yasmin and Yahya 1996) with males exhibiting a complex array of morphological traits, including feather ornaments and colors (Petrie et al. 1991), and behavioral displays. Train length and tarsus length are determinant in the process of territory establishment in the lek (intra-sexual selection) (Loyau et al. in press), and females use the number of ocelli in the train as well as display activity as cues during mate choice (inter-sexual selection) (Petrie et al. 1991; Petrie and Halliday 1994; Yasmin and Yahya 1996; Loyau et al. in press).

Ornaments, such as feathers, and behavioral traits can differ in their temporal pattern of variation. Feathers are fixed traits, which should contain information about the male past condition (at the time of their formation) rather than present condition (at the time of mate choice) (Sullivan 1994). Conversely, behavioral traits are sensitive to current condition and females assessing these traits might have access to information on the present phenotypic quality of the male at the time of mate choice (Sullivan 1994; Hill et al. 1999; Kokko et al. 1999). Traits with different developmental patterns might thus convey information on different temporal aspects of male quality (Wedekind 1992).

It is generally assumed that peahens can only gather indirect genetic benefits from mating with a particular male because peacocks do not provide any parental care (Petrie 1994). However, females might also directly benefit from their mate choice if copulating with a diseased male enhances the risk of contracting the disease (Borgia and Collis 1989; Able 1996; Loehle 1997). If females use feather ornaments and behavioral displays to avoid unhealthy males (Hamilton and Zuk 1982; Borgia and Collis 1989; Folstad and Karter 1992; Able 1996), one should expect that, in peacocks, both traits are related to individual health status (Verhulst et al. 1999; Møller and Petrie 2002; Faivre et al. 2003).

In this study, we wished to test the idea that multiple traits (feather ornaments and behavioral displays) might convey information on male health status in a free-ranging population of peacocks (*Pavo cristatus*). We predict that (i) both the expression of behavioral traits as well as ornaments should be correlated with current health status; (ii) if behavioral displays reliably reflect current health, experimental activation of the immune system with a bacterial antigen should reduce the intensity of such displays; (iii) if ornaments reflect genetic and/or long-term phenotypic quality, more ornamented males should be better able to cope with the simulated bacterial infection.

Methods

During the entire breeding season 2002, we monitored a free-ranging population of peafowl in the "Parc Zoologique de Clères" (Muséum National d'Histoire Naturelle, France), where birds are marked with numbered and colored metallic rings. A previous study conducted on this population has shown that several male traits are involved in the process of inter-sexual selection, as females use both the number of eyespots in the train and the male courtship display as cues during mate choice (Loyau et al. in press).

Between April 12th and May 15th, 2002 courtship displays of 24 territorial peacocks were monitored with a total of 105 h of focal observations (35 h per male, an average of 10 males were observed simultaneously). Observations were conducted between 0900 and 1700 with a mean duration of about 3 h per observation period. We recorded the number of sexual displays (i.e., males opening their train and exposing it to the female; see Petrie et al. 1991 and Loyau et al. in press). Each male was photographed with a digital camera (at a distance <6 m) with its train fully displayed and facing the camera. The pictures were then used to count the number of eyespots (Manning and Hartley 1991; Petrie et al. 1991) (for detailed method see Loyau et al. in press). Tail feathers molt once a year in August and keep growing during the fall and winter.

After this preliminary information was gathered, we captured each of these males, took a blood sample from the right jugular vein, made a blood smear and assigned individual males to two treatment groups in alternated order. Twelve peacocks received an intramuscular injection (4 ml) of LPS solution (1 mg/ml) (*E. coli* lipopolysaccharides serotype O55:B5, Sigma Chemical, St. Louis); 12 peacocks were injected with 4 ml of phosphate buffered saline (PBS) and served as a control. To check that groups were randomly formed we verified that there were no initial differences in either number of eyespots (one-way ANOVA, $F_{1,22}=0.01$, $p=0.932$), in display rate (one-way ANOVA, $F_{1,22}=2.38$, $p=0.138$), or in number of circulating heterophils (one-way ANOVA, $F_{1,22}=0.16$, $p=0.695$) between LPS and PBS individuals. Peacocks were immediately released after manipulation. LPS is a potent activator of both branches of the immune system as it induces an inflammatory response that is followed by the production of specific antibodies (Poxton 1995; Bonneaud et al. 2003). The number of sexual displays was subsequently monitored blindly with respect to the immune treatment, over a total of 120 h, during the 34 days following the activation of the immune system. Observations of sexual displays were a priori clustered into six periods (prior to the immune challenge, 1–4, 5–9, 10–14, 15–24, and 25–34 days after injection).

Health status

Health status was assessed using the number of heterophils per μl of blood, following the indirect method described by Campbell (1995). First, the proportion of heterophils

(%H) and eosinophils (%E) (out of 100 white blood cells) is assessed by screening smears with a light microscope at magnification $\times 100$. Second, the total number of granulocytes (heterophiles plus eosinophils) ($nH + nE$) is assessed using a Mallassez cell after a blood dilution of 200 with a 2% ammonium oxalate solution. By dividing the number of granulocytes by the proportion of heterophils and eosinophils, one therefore obtains the total number of white blood cells (TWBC) (1).

$$\text{TWBC} = \frac{(nH + nE)}{\%H + \%E} \times 200 \quad (1)$$

Finally, the number of heterophils per μl of blood can be obtained by multiplying the proportion of heterophils by the total number of white blood cells (2) (Campbell 1995).

$$\text{Number of heterophils per } \mu\text{l of blood} = \text{TWBC} \times \%H \quad (2)$$

Heterophils are released by the bone marrow in response to many types of inflammatory diseases, they are the first to arrive at the site of inflammation, and their number is medically used as an indication of infection (Campbell 1995; Goldsby et al. 2000). High numbers of heterophils are therefore indicative of poor health status.

Statistical analyses

The correlation between the number of heterophils and both behavioral display and number of eyespots was appraised using a multiple regression model. The effect of immune activation on display rate was assessed using a repeated measurement ANOVA. Data were analyzed using SAS (1999).

Results

A stepwise multiple regression showed that the frequency of sexual displays was negatively correlated with the number of circulating heterophils (slope \pm SE = -0.807 ± 0.310 , partial $R^2 = 0.289$, $p = 0.0167$, Fig. 1a); the number of eyespots was also negatively correlated with the number of heterophils, but the correlation was not significant (slope \pm SE = -0.011 ± 0.006 , partial $R^2 = 0.106$, $p = 0.066$; Fig. 1b), indicating that individuals with high display rates and possibly large eyespot numbers were in better health.

Activation of the immune system by the LPS significantly reduced the frequency of sexual displays (repeated measurements ANOVA: tests of hypotheses for between subjects effects, immune treatment, $F_{1,22} = 9.09$, $P = 0.0064$; tests of hypotheses for within subject effects, time, $F_{5,110} = 13.21$, $p < 0.0001$, time \times immune treatment, $F_{5,110} = 1.41$, $p = 0.225$). When looking at the temporal changes of display rate for the two groups of peacocks,

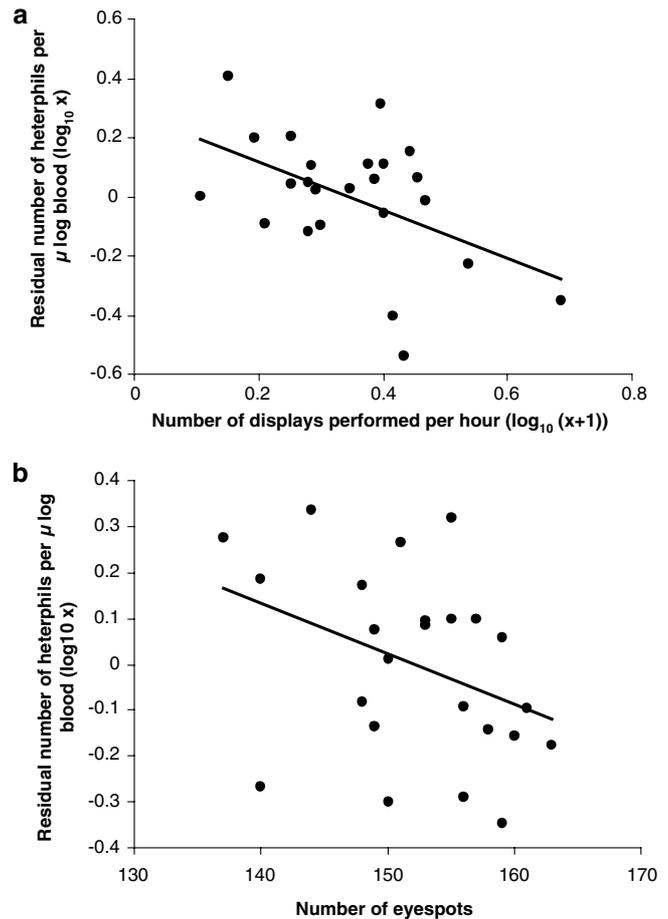


Fig. 1 a Negative correlation between residual number of circulating heterophils in the blood (number of cells/ μl of blood) and frequency of behavioral displays (number of displays per hour); b negative correlation between residual number of circulating heterophils in the blood (number of cells/ μl of blood) and number of eyespots in the tail. Residual number of circulating heterophils were obtained by regressing number of circulating heterophils on number of eyespots and frequency of behavioral displays, respectively

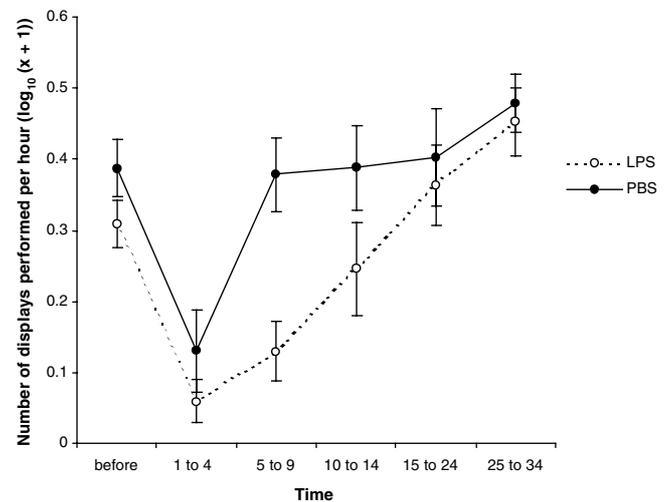


Fig. 2 Effect of immune activation (LPS vs. PBS) on frequency of behavioral displays. Bars represent standard errors

it appears that the manipulation of birds strongly affected their willingness to display (Fig. 2). However, 5 days after the manipulation, PBS birds completely recovered from the capture-induced stress and behaved as prior to the manipulation, whereas LPS birds took longer to recover (Fig. 2). These results therefore show that the activation of the immune system significantly reduced the expression of behavioral display in peacocks.

The condition-dependent theory of sexual advertisement predicts that the cost of the expression of sexual displays should be particularly high in poor quality individuals (Zahavi 1975; Grafen 1990). We might therefore expect that peacocks with large eyespot numbers were better able to cope with the immune challenge, and to maintain a high rate of behavioral display. To test this hypothesis we computed the LPS-induced proportional change in display rate (display rate 5–9 days after the immune challenge minus the display rate prior to the manipulation divided by the display rate prior to the manipulation) and correlated these values with the number of eyespots. We found that proportional changes in display rate were positively correlated with eyespot number ($r_s=0.637$, $n=12$, $P=0.026$); peacocks with the lowest number of eyespots suffering the strongest LPS-induced reduction in display rate (Fig. 3).

Discussion

Peahens have been shown to value multiple traits to choose a mate, the frequency of behavioral displays performed by males as well as the number of eyespots in the train (Loyau et al. in press). In this study, we found that the male display rate and the number of eyespots in the train can be used by females to predict male current health status. The LPS-induced reduction in display rate shows that this trait honestly reflects current health status. By using different traits, females might benefit from complementary information or from an enhanced accuracy of such infor-

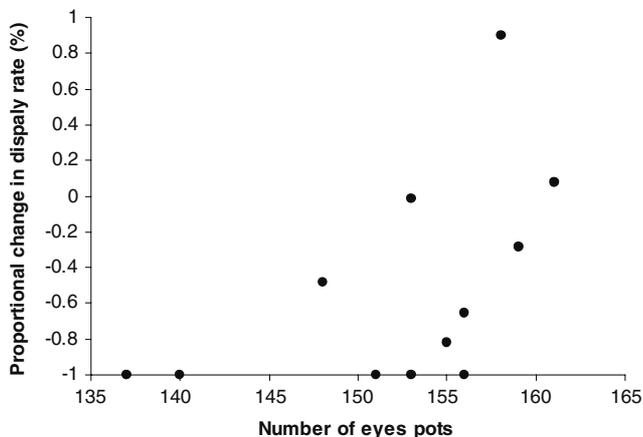


Fig. 3 Positive correlation between LPS-induced proportional change in display rate (display rate at 5–9 days post immune challenge minus display rate prior to the immune challenge divided by display rate prior to the immune challenge) and number of eyespots in peacocks

mation (Sullivan 1994; Rowe 1999). For instance, multiple traits might provide independent cues on the direct and indirect benefits females are likely to obtain when choosing a male (Buchanan and Catchpole 1997), or they could allow females to assess specific aspects of male resistance to parasites and choose mates with complementary genes (Wedekind 1992, 1994).

In peacocks, benefits of female preference are supposed to be restricted to good genes for the progeny because male contribution to the reproduction is limited to the transfer of genetic material (Petrie 1994). Peahens mated with more ornamented males have been shown to produce offspring that grow faster and survive better than offspring produced by females mated with less attractive males (Petrie 1994). Our study suggests that these good genes females seek might be linked to the immune system and the male ability to resist infectious diseases (Hamilton and Zuk 1982; Folstad and Karter 1992). Passed on the offspring these immune protection genes might explain why chicks sired by more ornamented peacocks have been shown to have enhanced growth and survival (Petrie 1994). In addition, we suggest that females can also obtain direct benefits through mate choice if the behavioral display of males honestly reflects health status. Avoidance of diseased partners could in this case limit the chance of contracting a sexually transmitted disease (Sheldon 1993).

Interestingly, consistent with the predictions of the handicap models of sexual selection (Zahavi 1975; Grafen 1990), males were not all affected in the same way by the immune activation. More ornamented males, as assessed by the number of eyespots in the tail, were better able to cope with the simulated infection and maintained similar levels of display rate as prior to the immune activation. Similarly, male collared flycatchers (*Ficedula albicollis*) whose immune system was activated showed a reduction in song rate, but the effect was modulated by the size of the forehead patches and by song complexity (Garamszegi et al. 2004). These studies therefore show that males of higher phenotypic/genetic quality undergo lower detrimental effects of the activation of the immune system, suggesting that the costs of immune functioning are lower in high quality individuals.

Morphological and behavioral traits differ in their temporal pattern of variation and therefore may reflect male quality over different time scales (Scheuber et al. 2003). Ornaments might be long-term signals that advertise male past quality at the time of feather formation (Sullivan 1994) (e.g., in peacocks, tail feathers are molted in summer and grow during winter). Conversely, behavioral displays can be shaped by short-term environmental factors and can respond rapidly to variations in health status. Fixed traits may more reliably reflect the genetic quality of males, whereas flexible traits may indicate current condition (Hill et al. 1999; Kokko et al. 1999). In the context of mate choice, females could gain indirect benefits in terms of “good genes” for their progeny by assessing long-term signals (Scheuber et al. 2003) and simultaneously could obtain direct benefits by appraising short-term signals. However, more work is clearly needed in peafowl to further investigate this issue.

Usually, once produced, male secondary sexual ornaments (e.g., bright colored feathers, antlers, comb) are permanently assessable to females during the entire breeding season, whereas behavioral courtships are signals that can be switched 'on' and 'off' depending on the male motivational level and sexual interest (Hauser 1996; Kodric-Brown and Nicoletto 2001). In the peacock, the number of eyespots in the train can only be assessed by females when male spread their trains during the courtship display. In this species, the expression of the ornament is modulated by the expression of the behavior. Similarly, shape and color of the eyespots can enhance courtship display detection and assessment (Rowe 1999). Given that more ornamented males were better able to maintain high display rates when experimentally challenged, differences in male health status are probably enhanced by the interaction between these two quality-indicator signals improving the discrimination of male quality (Hasson 1989; Fitzpatrick 1998). Therefore, both morphological and behavioral traits may reinforce each other to influence female choice, each trait amplifying the message conveyed by the other trait, thereby enhancing information reliability (Hasson 1989; Candolin 2003).

In conclusion, using multiple traits with different pattern of temporal variation might be valuable for females as this might give access to information on past and current mate condition, and this phenomenon may be more widespread than previously acknowledged since in many species males harbor fixed as well as flexible sexual traits.

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