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Sexual colouration and sperm traits in guppies

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The relationships among the area, hue, saturation and brightness of orange colouration and sperm traits in the guppy *Poecilia reticulata* were investigated. Males with greater areas of orange colouration had significantly larger sperm loads, more motile sperm and longer sperm relative to males with relatively little orange colouration. Males with greater areas of orange colouration did not possess more viable sperm than males with relatively little orange colouration. There was no evidence that any of the sperm traits were related to the hue, saturation or brightness of the orange colouration. These results are discussed in the context of the roles that direct and indirect selection might play in maintaining female preference for male guppies with large areas of orange colouration.

Key words: fertility; good genes; guppy; mate choice; sexual ornamentation; sperm.

INTRODUCTION

One of the basic assumptions of evolutionary ecology was that 'sperm is cheap' and, therefore, all males can provide a sufficient supply of high quality sperm at each mating (Bateman, 1948; Trivers, 1972). Increasingly, however, this assumption has been challenged by evidence suggesting that (1) sperm production can be costly (Dewsbury, 1982; Olsson *et al.*, 1997), (2) males prudently allocate sperm during mating (Wedell *et al.*, 2002) and (3) substantial within-species variation in sperm production and quality is common (Sheldon, 1994; Matthews *et al.*, 1997; Engen & Folstad, 1999). Males may either produce poor quality sperm or become functionally infertile by failing to produce enough sperm to successfully fertilize a female's entire compliment of eggs (Nakatsuru & Kramer, 1982; Shapiro *et al.*, 1994; Malo *et al.*, 2005*a*). Thus, sexual selection theory predicts that females should favour those males that can supply sufficient quantities of high quality sperm. This prediction is the premise for the phenotype-linked fertility hypothesis (Sheldon, 1994), which suggests that male secondary sexual characters can provide females with cues about male

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fertilizing ability (Trivers, 1972). This hypothesis has been of interest recently (Skarstein & Folstad, 1996; Liljedal *et al.*, 1999; Peters *et al.*, 2004; Pizzari *et al.*, 2004; Malo *et al.*, 2005*b*) because it offers one explanation, based on direct rather than indirect selection (Neff & Pitcher, 2005), for the maintenance of female preference for male ornaments in non-resource based mating systems (Sheldon, 1994; Blount *et al.*, 2001).

The purpose of the present study was to investigate the relationship between the area, hue, saturation and brightness of males' orange sexual colouration and sperm traits in the guppy *Poecilia reticulata* (Peters). Guppies are live-bearing fish with internal fertilization and a promiscuous mating system (Houde, 1987, 1997), where females from many populations prefer males with more intense hue and saturation of their orange colouration (Kodric-Brown, 1989; Houde & Torio, 1992; Grether, 2000) and a greater area of orange colouration (Houde, 1987; Endler & Houde, 1995; Pitcher *et al.*, 2003; Evans *et al.*, 2004*a*). The hue and saturation of male orange colouration is probably related to some extent to carotenoid reserves because studies have shown that the level of carotenoids in the diet affects these metrics (Kodric-Brown, 1989; Grether, 2000). Carotenoids play roles in male reproductive function in ways that can influence sperm quality and quantity (vonSchantz *et al.*, 1999; Blount *et al.*, 2001). Carotenoids, for example, are present in gonads, reproductive accessory glands and seminal plasma (Goodwin, 1984; Aitken, 1995; Heller *et al.*, 2000). These pigments act as antioxidants functioning to intercept and neutralize free radicals, which have a deleterious effect on sperm quality (Halliwell & Gutteridge, 1999; vonSchantz *et al.*, 1999). Accordingly, antioxidants present in semen appear to reduce the susceptibility of sperm to oxidative stress and increase fertility (Blount *et al.*, 2001). In contrast to hue and saturation, the area of orange colouration found on male guppies does not appear to be affected by carotenoid intake (Kodric-Brown, 1989; Grether, 2000) but instead has a substantial genetic component (Houde, 1992). Area of orange colouration in guppies has been shown to be negatively related to the level of inbreeding (van Oosterhout *et al.*, 2003), which is associated with reduced sperm quantity or quality in many taxa (Wildt *et al.*, 1987; Roldan *et al.*, 1998; Margulis & Walsh, 2002).

The underlying premise of the phenotype-linked fertility hypothesis is that females will receive higher quality sperm from more attractive males (Sheldon, 1994). This hypothesis requires that male sexual ornaments that females prefer are honest indicators of sperm quality. Ornamentation can honestly advertize sperm quality *via* condition dependence as originally suggested by Sheldon (1994). Ornaments may also honestly advertize sperm quality if they are genetically determined, and reveal genetic load associated with inbreeding, which can have deleterious effects on sperm quality (Wildt *et al.*, 1987; Roldan *et al.*, 1998; Margulis & Walsh, 2002). An earlier study on guppies suggested that sperm number was an increasing function of the area of orange colouration for males from one, but not a second, population, but that study did not examine sperm motility, viability or morphology (Pitcher & Evans, 2001). To test whether orange colouration in male guppies signals sperm quality and thus their potential fertilization capacity (Billard *et al.*, 1995*a*; Rurangwa *et al.*, 2004), the hue, saturation, brightness and area of orange colouration were assessed and related to sperm number, motility, morphology and viability.

MATERIALS AND METHODS

EXPERIMENTAL FISH

Guppies were collected from a tributary of the Paria River in the Northern Range, Trinidad (10°44′42″ N; 61°15′42″ W). This is a low-predation locale where guppies coexist with the gape-limited cyprinodontid fish *Rivulus hartii* (Boulenger) and the freshwater prawn *Macrobrachium crenulatum*. Males from this Paria tributary are highly ornamented, with large areas of the body covered with orange, and females from this population prefer males with more orange (area, hue and saturation of orange colouration; Houde, 1987; Houde & Torio, 1992; Pitcher *et al.*, 2003).

All males used in this study ($n = 37$) were sexually mature, based on gonopodium morphology (Houde, 1997). Males were collected in the wild and held in the laboratory for <10 days before being tested. They were fed twice daily with Tetramin® flake food, which contains some carotenoids. Males were separated from females 3 days prior to being examined for colour patterns and sperm features to ensure full size sperm loads (Pilastro *et al.*, 2002). Males were then anaesthetized in a water-bath containing neutralized 0·15 g l^{-1} MS 222, weighed (to the nearest 0·01 g), photographed and then placed on a Petri dish under a dissecting microscope in order to collect sperm.

ORANGE COLOUR ASSESSMENT

To estimate the hue, saturation, brightness and area of orange colouration, males were photographed using a digital camera (Nikon CoolPix 950®) with a ruler under standardized light conditions, consisting of two fibre optic light sources placed 10 cm above the male's body surface with ambient room lighting from incandescent bulbs. In order to ensure that lighting conditions were standardized for each guppy, pictures were taken of a colour palette (paint chips) prior to each photograph; the brightness of the colour palette was then measured using Adobe Photoshop®.

To estimate the intensity of orange colouration on males, digital images were measured using Adobe Photoshop® to produce numerical estimates of three colour properties: hue, saturation and brightness (Skarstein & Folstad, 1996; Liljedal *et al.*, 1999). Hue is the property commonly referred to as colour, which indicates the dominant wavelengths of light reflected from an object. In Adobe Photoshop®, hue is measured as an angular location on a standard colour wheel (expressed as a number between 0 and 360 degrees). In common use, hue is identified by the name of the colour such as orange [range: *c.* 15 degrees (reddish-orange) to *c.* 45 degrees (yellowish-orange)]. Saturation is the strength or purity of the colour ('chroma'). Saturation represents the amount of grey in proportion to the hue, measured as a percentage from 0% (grey) to 100% (fully saturated) (Long & Luke, 2001). An individual is considered more ornamented if its colour has a more reddish-orange hue (*i.e.* hue score closer to 15 degrees) and is more saturated. Brightness is the relative lightness or darkness of the colour, measured from 0 (black) to 100% (white).

Hue, saturation and brightness were each estimated at three different points equidistant along a horizontal transect through the middle of each orange spot on each male, and the mean value for each spot was calculated. Afterwards, the three colour properties were averaged for each male for all of the orange spots on his body (see Table I). The area of orange colouration, total surface area of the body (including head and spread out tail fin) and standard length (L_S) were also quantified from the digital images using NIH Image analysis software (available at http://rsb.info.nih.gov/nih-image/).

SPERM TRAIT ASSESSMENT

Immediately after digital photographs were taken, following Matthews *et al.* (1997), sperm was collected by placing the male under a dissecting microscope and swinging the gonopodium forward. Slight pressure was then applied to the side of the abdomen,

TABLE I. Body size, colour and sperm related variables in male guppies (mean ± S.E. for untransformed data, $n = 37$ males)

Variable	Mean ± S.E.	Range
L_S (mm)	14·9 ± 0·2	12·7–17·2
Orange area (%)	16·1 ± 0·8	8·0–28·1
Hue (°)	30·6 ± 0·7	24·1–43·3
Saturation (%)	75·5 ± 0·7	64·9–83·7
Brightness (%)	94·4 ± 0·8	80·7–99·9
Motility (μm s^{-1})	48·5 ± 9·1	35·3–67·9
Viability (%)	92·7 ± 3·2	86·7–99·3
Number ($\times 10^6$)	3·3 ± 0·3	1·0–7·3
Head length (μm)	8·9 ± 0·9	6·9–11·2
Flagellum length (μm)	41·2 ± 3·1	35·8–49·5

L_S, standard length.

just anterior to the gonopodium, to release the spermatozeugmata (*i.e.* sperm bundles). Pressure was applied repeatedly until all sperm bundles were extracted. Sperm bundles were then drawn up in a pipette and added to 250 μl of Courtland's saline solution, which contained bovine serum albumin at 1% v/v (hereafter saline solution). To break up the sperm bundles and release the sperm into solution, samples were gently mixed by repeatedly drawing up and expelling the sample from the pipette. Once bundles were broken up, sperm motility, viability, number and morphology was examined, respectively.

Sperm motility was assessed for each male with a digital compound microscope (magnification ×400, Olympus BX60) by viewing an 8 μl sample of semen in saline solution on a slide, covered with a cover slip. Sperm motility for each male was quantified as a rate by measuring the linear path distance a sperm travelled (μm) before leaving the field of view and dividing that by the time (s) it was tracked. To reduce the time till the sperm stuck to the glass, the glass slide and cover slip were pre-coated by immersion in 1% bovine serum albumin followed by a rinse in distilled water (Billard *et al.*, 1995*b*). Sperm that were stuck to one another or the glass slide and those whose movement beneath the cover slip was caused by convection currents were excluded from analyses. The amount of time (rounded to the nearest 0·5 min, mean ± S.E. = 3·3 ± 0·2 min, range: 2·5–5 min) from when the sperm were collected until when motility was assessed were recorded to determine if this duration affected the measurements. Between 12 and 26 sperm were measured per male (mean ± S.E. = 20·6 ± 0·7). Differences in the number of sperm examined per male resulted from differences in the number of appropriate sperm that could be found in the first moments after applying the sperm solution to the glass slide.

Sperm viability was investigated using a sperm viability assay (Rurangwa *et al.*, 2004), where viability refers to the integrity of the sperm membranes. Twenty μl of sperm suspension was placed on a glass slide immediately after sperm were removed from the male and 2 μl of trypan blue stain was added. The two solutions were mixed with a sterile probe and smeared along the slide using another sterile slide. The smear was covered with a cover slip and examined under a microscope at ×1000 under oil immersion *c.* 1 min after motility measurements were taken for each sample to allow the stain time to permeate the cells. Live sperm cells with intact membranes appear colourless whereas sperm cells with disrupted membranes (*i.e.* dead) were stained. The number of live and dead sperm were counted in three groups of 100 sperm per male at different locations on the slide and mean of these three measures were used for all analyses (see Table I).

Sperm counts were calculated by counting sperm cells in an 'improved Neubauer chamber' haemocytometer under ×400 magnification. The distribution of sperm cells across the haemocytometer was checked visually for evenness before counts commenced. If the sperm were unevenly distributed across the haemocytometer then the count was

discarded and started over again. The numbers of sperm in each of five larger squares on the haemocytometer were counted. There are 25 of these large squares on the haemocytometer and each of these large squares has 16 smaller squares within it. Sperm were counted in the four large corner squares and the large centre one (80 smaller grids). The mean number of sperm per large square count (*i.e.* mean of the five counts) was multiplied by 25 (to obtain the mean per 5×5 large square grid) and again by 10 (the depth of the chamber in μm). This number was then multiplied by the initial volume of the sample to estimate the sperm load. Sperm counts are expressed as the total number of sperm in a male's stripped ejaculate (see Table I).

A sub-sample of sperm (20 μl) was used to assess sperm morphometry using techniques similar to those described by Leach & Montgomerie (2000). Sperm in saline solution were dispensed onto a glass slide to present two-dimensional images for measurement. Sperm were observed at $\times 1000$ magnification under oil immersion and digital images were taken (with a scale provided by the digital scope). The head and flagellum were measured separately (the midpiece was too small to discern using light microscopy and was indistinguishable from the head component) using NIH image software and care was taken to only measure intact sperm without flagellar damage. Head length (which included the midpiece) was the measurement from the insertion of the flagellum across the midline of the sperm head to its forward apex; flagellum length was measured from its insertion to the end of the terminal filament. Total sperm length was determined by combining the head length and the flagellum length. Twenty-five sperm per male were measured and the mean of the measurements taken on total, head and flagellum length were used in all analyses (see Table I).

STATISTICAL ANALYSES

Because the area of orange colouration on males was a function of their body area ($r = 0{\cdot}48$, d.f. $= 36$, $P < 0{\cdot}01$), the relative area of orange was used as an index of how colourful males were. Relative area of orange colouration (hereafter area of orange colouration) was estimated from the residuals of the regression of the $\log_{10}$ of orange area on the $\log_{10}$ of total area of the body. Hue, saturation and brightness were not related to body area or L_S (all $P > 0{\cdot}05$). The hypothesis that sperm features (number, motility and viability) were related to the hue, saturation, brightness or area of orange colouration was tested using linear regressions. To control for potentially confounding effects, male L_S and condition (the residuals of the regression of body mass and L_S) were entered in multiple regression analyses of sperm number. To control for potentially confounding effects, male L_S, condition and time since sperm were collected were entered in the multiple regression analyses of sperm motility and viability. The relationship between motility and total sperm length was examined using a Pearson correlation (r). Multiple linear regression was also used to determine whether head length or flagellum length could further explain variation in motility. In all analyses, regressions were performed using motility and viability means for each male as independent data points to avoid pseudoreplication.

All means are reported ±S.E. All proportional data (*i.e.* sperm viability and orange saturation) were arcsine square root transformed prior to statistical analyses in order to render them normally distributed (Zar, 1996). In addition, sperm number, length, motility and hue data were $\log_{10}$ transformed prior to statistical analyses in order to meet the assumptions of the parametric tests (Zar, 1996). All statistical tests were performed using general linear models (GLM) and correlation analyses in SPSS (version 12).

RESULTS

Area of orange colouration was a significant predictor of sperm number [$r^2 = 0{\cdot}27$, $P = 0{\cdot}001$, $n = 37$; Fig. 1(a)] and sperm motility [$r^2 = 0{\cdot}22$, $P < 0{\cdot}01$, $n = 37$; Fig. 1(b)], but was not a significant predictor of sperm viability [$r^2 = 0{\cdot}05$, $P > 0{\cdot}05$, $n = 37$). These relationships remained significant when

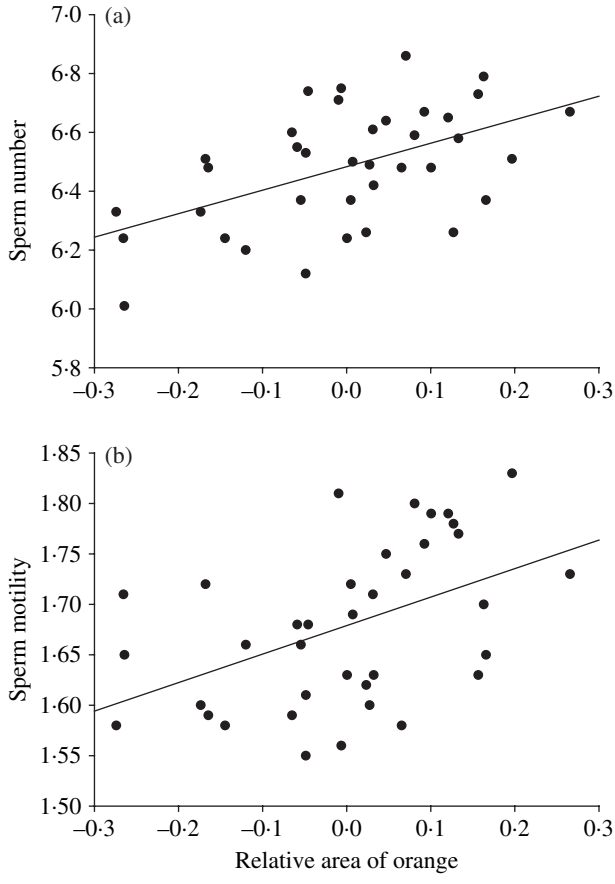

FIG. 1. Relationship between relative area of orange colouration and sperm (a) number ($\log_{10}$ transformed) and (b) motility ($\log_{10}$ transformed). The curves were fitted by (a) $y = 0{\cdot}79x + 6{\cdot}48$ and (b) $y = 0{\cdot}28x + 1{\cdot}68$.

potentially confounding variables were controlled for by using multiple linear regressions (see Table II). There was a significant correlation between area of orange colouration and total sperm length ($r = 0{\cdot}50$, $P < 0{\cdot}01$, $n = 37$). From a mechanistic perspective, a multiple linear regression was used to assess whether head length or flagellum length was responsible for the relationship between area of orange colouration and motility. There was a significant relationship between motility and the length of the sperm's head, but not the flagellum's absolute (Table III and Fig. 2) or relative length ($P > 0{\cdot}05$). Hue, saturation and brightness of orange colouration were not significant predictors of sperm number (all $P > 0{\cdot}05$), motility (all $P > 0{\cdot}05$) or viability (all $P > 0{\cdot}05$).

DISCUSSION

The results of this study support the premise of the phenotype-linked fertility hypothesis because elements of orange ornamentation appear to be indicators of sperm traits in guppies. The area of orange colouration was associated with

TABLE II. Results from linear multiple regressions with sperm number, motility and viability as the dependent variables. Independent variables tested were relative orange area (residuals of the regression of the $\log_{10}$ of orange area on the $\log_{10}$ of total area of the body), male standard length (L_S), condition and time since sperm were extracted from the male (for the motility and viability analyses)

Sperm number		
Source*	β	*P*
Orange area	0·45	0·005
L_S	0·23	0·13
Condition	0·11	0·47

*Overall model: $r^2 = 0{\cdot}31$, $F_{3,33} = 4{\cdot}87$, $P < 0{\cdot}01$.

Sperm motility		
Source*	β	*P*
Orange area	0·44	0·008
L_S	0·06	0·70
Condition	0·19	0·23
Time	0·16	0·32

*Overall model: $r^2 = 0{\cdot}27$, $F_{4,32} = 2{\cdot}91$, $P < 0{\cdot}05$.

Sperm viability		
Source*	β	*P*
Orange area	−0·23	0·16
L_S	0·35	0·04
Condition	−0·19	0·25
Time	−0·11	0·52

*Overall model: $r^2 = 0{\cdot}20$, $F_{4,32} = 2{\cdot}05$, $P > 0{\cdot}05$.
β, The standardized regression coefficient, which reflects the direction and magnitude of the effect; *P*, corresponding significance.

both sperm number and motility. The hue, saturation and brightness of orange colouration, however, were not associated with any of the sperm traits that were measured. These results suggest that female guppies may benefit from mating with males with a larger area of orange colouration, because sperm

TABLE III. Results from a linear multiple regression with sperm motility as the dependent variable. Independent variables tested were head size and flagellum size

Source*	β	*P*
Head length	0·33	0·03
Flagellum length	0·26	0·10

*Overall model: $r^2 = 0{\cdot}20$, $F_{2,34} = 4{\cdot}11$, $P < 0{\cdot}05$.
β, The standardized regression coefficient, which reflects the direction and magnitude of the effect; *P*, corresponding significance.

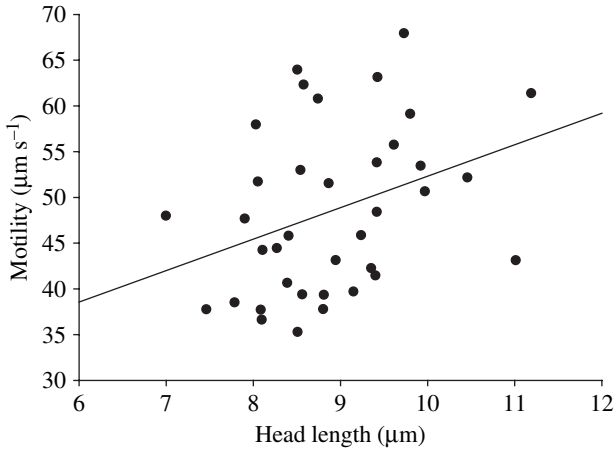

FIG. 2. Relationship between sperm head length and motility. The curve was fitted by $y = 3{\cdot}84x + 8{\cdot}91$.

motility and sperm number can both influence a male's fertilizing capacity (Billard *et al.*, 1995*a*; Evans & Magurran, 2000; Bayley *et al.*, 2003; Evans *et al.*, 2003; Rurangwa *et al.*, 2004). Indeed, females from this population do prefer males with larger areas of orange colouration (Houde, 1987; Endler & Houde, 1995; Pitcher *et al.*, 2003).

Consistent with a previous study (Pitcher & Evans, 2001), a significant percentage (27%) of the variation in sperm number was predicted by the area of orange colouration for males in this population [Fig. 1(a)]. Interestingly, the relationship between area of orange colouration and sperm number in this Paria population was not evident for males from another population, the Tacarigua River (Pitcher & Evans, 2001). In that high-predation population, where females also prefer males with more orange colouration (Evans *et al.*, 2004*b*), males with more carotenoid colouration did not possess significantly larger sperm loads. Instead, in the Tacarigua population, there was significant relationship between male display rate and sperm number (Matthews *et al.*, 1997).

In the present study, a second component of fertilizing efficiency, the motility of sperm, was also related to the area of orange colouration [Fig. 1(b)]. Likewise, males with larger areas of orange colouration had significantly longer sperm; this observation provides a possible explanation for the greater motility of sperm from males with larger orange areas (Gomendio & Roldan, 1991). When the overall sperm length was separated into head and flagellum length components, however, it was found that head length explained a significant amount of variation in motility whereas flagellum length did not (see Fig. 2 and Table III). Adenosine triphosphate (ATP) hydrolysis is required to maintain flagellar motility and sperm ATP is mainly stored in the midpiece (Christen *et al.*, 1987; Billard *et al.*, 1995*a*; Zilli *et al.*, 2004). Thus, the size of the midpiece may be indicative of ATP content because this section contains the densely packed array of mitochondria that provides energy for sperm motility (Baccetti & Afzelius, 1976). Interestingly, the midpiece is very pronounced in guppies and represents a large percentage of head size (Billard *et al.*, 1995*a*). Unfortunately, the midpiece could not be distinguished from head size

using light microscopy. Future studies should assess midpiece size using transmission electron microscopy of longitudinal sections of guppy sperm.

It is not obvious why there are associations between area of orange colouration and sperm number and motility. First, it is possible that there is a genetic correlation between the area of orange colouration and these sperm traits. This relationship could arise if genes for area of orange colouration and genes for sperm production were in linkage disequilibrium, or because they are both pleiotropic effects of some shared underlying genes. The area of orange colouration in guppies is *Y*-linked in the present study population (Houde, 1992) and may, therefore, be associated with sperm related genes (Roldan & Gomendio, 1999). A link between sperm features (*i.e.* motility and number) and orange colouration area on guppies may also be mediated by genetic load arising from inbreeding depression (van Oosterhout *et al.*, 2003). Sperm traits have a genetic basis in many taxa (Woolley & Beatty, 1967; Morrow & Gage, 2001) and sperm abnormalities have often been associated with inbreeding depression (O'Brien *et al.*, 1985; Wildt *et al.*, 1987; Roldan *et al.*, 1998; Gomendio *et al.*, 2000; Margulis & Walsh, 2002).

It is also probable that some of the association between area of orange colouration and sperm production in guppies is developmental. Differences in sperm number and quality have been found in relation to ontogeny in many taxa (Calvo *et al.*, 1999; Ceballos-Vazquez *et al.*, 2003; Green, 2003). Male guppies from this Paria tributary begin to develop their orange colouration before they are sexually mature (some orange spots have reached *c.* 60% of final size at sexual maturity), but the area of orange does not attain its full size until 22–25 days after maturity in the laboratory (L. Bannister & H. Rodd, unpubl. data). Evans *et al.* (2002) also found that the area of orange colouration increased with age in males from the Tacarigua River. As such, in the present study, the relationship between area of orange colouration and sperm number may well result, at least in part, from differences in the stage of development of the males examined; other than knowing that the males were sexually mature based on gonopodium morphology (Houde, 1997), there was no other way of ageing the males and therefore this hypothesis could not be evaluated directly.

The results of this study did not provide support for the idea that the hue, saturation or brightness of orange colouration might indicate sperm number, viability or motility (Blount *et al.*, 2001). There are at least four plausible explanations for these findings. First, the level of orange pigmentation in guppies may not be directly linked to the carotenoid related antioxidant resources that are presumed to affect sperm quality (Blount *et al.*, 2001). For example, Hartley & Kennedy (2004) suggest that carotenoid related colour displays may not be a direct display of antioxidant wealth because most potent antioxidant agents are actually colourless. Hartley & Kennedy (2004) also suggest that carotenoid related antioxidant properties may play a vital role in areas other than sperm quality, such as immune function or developmental processes (Houde & Torio, 1992; Grether *et al.*, 2004).

Second, it is possible that the measurements of orange hue, saturation and brightness used in this study were not good indicators of carotenoid content because other pigments are also involved in determining the level of orange intensity in male guppies. Grether *et al.* (2001) found that the orange coloured

spots that male guppies display to females contain not only carotenoid pigments, but also pterins (specifically drosopterins, pigments with similar spectral and antioxidant properties to carotenoids), which are displayed in the skins of many fishes, lizards and amphibians.

Third, although the wild caught guppies used in this experiment were housed in the laboratory for no more than 10 days prior to examination, this time in captivity may have reduced variation among males in carotenoid content (and orange colouration saturation and hue) below those that would be observed in the field because they were fed flake food containing some carotenoids in the laboratory.

Finally, the measurements of hue, saturation and brightness may not have been sensitive enough to detect differences among males. Unlike some studies that examined absorption spectra directly (Hudon *et al.*, 2003; Peters *et al.*, 2004), the measurements of orange colour hue and saturation used in this study were based on indirect estimates from digital images [other studies that have found significant variation among males in the same spectral properties examined in this study include Skarstein & Folstad (1996) and Liljedal *et al.* (1999)]. As such, the measure of hue, saturation and brightness used may not be as reliable as the measurements of area of orange colouration. In order to more accurately examine differences in the hue, saturation and brightness of orange colouration among guppy males, future studies could manipulate the amount of carotenoids given to full-siblings during development (Grether, 2000). This experimental design would allow the hue and saturation of orange colouration to be manipulated and the effects of genes and the environment on sperm number and motility to be disentangled.

The roles that direct and indirect selection play in maintaining preference of female guppies for males with large areas of orange colouration remain unclear (Houde, 1992; Evans *et al.*, 2004*b*). Some results suggest that brood size may be affected by the number of sperm a female receives during mating (Evans & Magurran, 2000; Bayley *et al.*, 2003). Sperm limitation in guppies could be relevant where mating is costly and where males, as in this population, begin to develop their colouration before they are sexually mature. In the wild, sperm limitation, however, may be uncommon because female have been shown to actively limit the sperm transferred during copulation (Pilastro *et al.*, 2004) which would be unexpected if sperm were limiting. With respect to indirect selection, Evans *et al.* (2003) found that when females were artificially inseminated with equal numbers of sperm from two male guppies, males with the greater area of orange colouration had higher paternity than those with smaller areas. Assuming that guppy sperm traits are heritable to some degree, sons may inherit good sperm competitive genes when females mate with more colourful males.

In conclusion, in guppies from the population studied here, sperm number, sperm length and motility were related to the area of orange colouration. If the relationship between sperm features and sexual ornamentation observed in this study is widespread in nature, it may help provide an explanation for the evolution and maintenance of female preference for extravagant male ornaments in non-resource based mating systems.

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