Dense, small and male-biased cages exacerbate male–male competition and reduce female choosiness in *Bicyclus anynana*

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Sexual selection is increasingly recognized to depend upon, and to fluctuate with, major ecological factors in natural environments. The operational sex ratio (OSR) and population density can affect the opportunity for, and strength of, sexual selection but their effects are rarely taken into account in laboratory behavioural studies. In *Bicyclus anynana*, a model butterfly for mate choice studies, the experimental set-up widely used in laboratories often involves very high densities compared to the field, male-biased sex ratios and small cage volumes. We hypothesized that these conditions impede the proper expression of female mating preference by promoting male competition and sexual harassment of females. Using various cage volumes, we separately manipulated OSR and density to cover the range of values used in *B. anynana* laboratory mating experiments and to approach field values. Male competition, quantified by the number and duration of courtships aborted by males, became stronger with increasing densities, specifically under more male-biased sex ratios, and decreasing cage volumes. Thus, male eagerness to mate was essentially due to the use of small cage volumes and very high experimental densities. Concomitantly, female choosiness, quantified by the proportion of rejections of male mating attempts, decreased with increasing densities under a male-biased sex ratio. Females also accepted more matings with decreasing cage volume, and mated more rapidly with increasing density. We conclude that the laboratory social environments frequently used to test mate choice in *B. anynana*, with unnaturally high densities and male-biased sex ratios, exacerbate male–male competition and strongly hinder female choice, biasing the estimates of the strength and direction of sexual selection that were shaped under very different natural environments in the wild. We propose a set-up for *B. anynana* mate choice studies that allows the assessment of female choosiness in statistically robust mating experiments.

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Understanding the processes of pair formation is a necessary, yet neglected, step to fully grasp how secondary sexual traits and mating preferences evolve under sexual selection. It requires taking into account the fact that sexual selection, like natural selection, takes place in ecologically complex environments and examining the two major selective agents of sexual selection, mate choice and within-sex competition, in an integrative way (Miller & Svensson, 2014). It is increasingly acknowledged that mating patterns depend on ecological and social constraints, which determine the availability of potential mating partners and, as a consequence, affect the expression of within-sex competition for mate access and of between-sex mating preferences (Miller & Svensson, 2014; Widemo & Saether, 1999). Social constraints include population density (defined here as the number of individuals ready to mate in a given space) and the operational sex ratio (OSR, i.e. the ratio of sexually active males to fertilizable females; Emlen & Oring, 1977; Kvarnemo & Ahnesjö, 1996). Sexual selection has been shown to be affected in various ways by population density (Crowley et al., 1991; Eshel, 1979; Hubbell & Johnson, 1987; Kokko & Rankin, 2006) and OSR (Clutton-Brock & Parker, 1992; Emlen & Oring, 1977; Kokko, Klug, & Jennions, 2012; Kvarnemo & Ahnesjö, 1996; but see Head, Lindholm, & Brooks, 2007). On the one hand, the effects of OSR and density on competition for mate access are relatively straightforward: mate competition usually increases with density (e.g. crickets, *Gryllus integer*: Cade & Cade, 1992; guppies,

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Poecilia reticulata: Jirotkul, 1999b; soldier beetles, *Chauliognathus pennsylvanicus*: McLain, 1982) and with increasing proportions of the mate-limited sex (usually males; e.g. lobsters, *Homerus gammarus*: Debuse, Addison, & Reynolds, 1999; Japanese medaka, *Oryzias latipes*: Grant, Bryant, & Soos, 1995; sand gobies, *Pomatoschistus minutus*: Kvarnemo, Forsgren, & Magnhagen, 1995; agile frogs, *Rana dalmatina*: Lodé, Holveck, Lesbarrères, & Pagano, 2004), which should lead to stronger sexual selection in the mate-limited sex, namely to higher variance in mating success or few individuals monopolizing more matings (OSR, Croshaw, 2010; Jones, Arguello, & Arnold, 2004; Klemme, Ylönen, & Eccard, 2007; density, Kokko & Rankin, 2006). On the other hand, the effects of OSR and density on mating preference, and in particular on choosiness (i.e. the effort and energy devoted to mate assessment), are more complex (Jennions & Petrie, 1997; Widemo & Saether, 1999; Wong & Candolin, 2005). An OSR biased towards the mate-limited sex (i.e. usually increasing proportions of males) and high density may decrease choosiness of the choosing sex (density, Arnqvist, 1992; Rowe, 1992; OSR, Lauer, Sih, & Krupa, 1996), via mating costs such as increased harassment (Thornhill & Alcock, 1983) or sexual interference (Schwagmeyer & Brown, 1983), and often correspond to frequent multiple mating (Lodé, Holveck, & Lesbarrères, 2005; Lodé et al., 2004; Rowe & Arnqvist, 2002; Rowe, Arnqvist, Sih, & Krupa, 1994; Uller & Olsson, 2008; but see these empirical studies: Laloi, Richard, Lecomte, Massot, & Clobert, 2004; Fitze, Le Galliard, Federici, Richard, & Clobert, 2005; and these modelling studies: Bleu, Bessa-Gomes, & Laloi, 2012; Härdling & Kaitala, 2005). As such, a male-biased OSR and high density can decrease the opportunity for, and strength of, sexual selection as the choosing sex then mates more indiscriminately (e.g. OSR, Arnqvist, 1992; Kokko et al., 2012; Krupa & Sih, 1993; density, Jirotkul, 1999b; Mills & Reynolds, 2003; Pomfret & Knell, 2008; Rowe et al., 1994). The reverse may also be true: choosiness may increase with increased OSR biases (Berglund, 1994; Gwynne & Simmons, 1990; Jirotkul, 1999a; Lawrence, 1986; Souroukis & Murray, 1995) and density (Gwynne, 1994; Palokangas, Alatalo, & Korpimäki, 1992; Shelly & Bailey, 1992), for instance via increased variance in quality of mates to choose from (Owens & Thompson, 1994) or decreased cost of mate searching facilitating mate quality assessment (Crowley et al., 1991; reviewed in Kokko & Rankin, 2006), which should strengthen sexual selection. In addition, sex roles can be reversed when the OSR is biased towards the choosing, and not towards the competing, sex (Clark & Grant, 2010; Forsgren, Amundsen, Borg, & Bjelvenmark, 2004; Gwynne & Simmons, 1990).

As shown above, OSR and density affect the opportunity for, and strength of, sexual selection. Yet, there are two main caveats in most studies published so far. First, the effects of both social constraints are likely to be entangled as variation in OSR depends on the relative densities of the sexes. One thus needs to manipulate OSR and density separately to be able to predict the consequences of their potentially complex interactions on mate competition and mating preference. So far, few empirical studies have attempted to do so both in invertebrates (Alonso-Pimentel & Papaj, 1996; Arnqvist, 1992; Janowitz & Fischer, 2012; Saeki, Kruse, & Switzer, 2005; Smith, 2007; Wang, He, Yang, Hedderley, & Davis, 2009; Wang, Yang, & Hedderley, 2008) and in vertebrates (Aronsen, Berglund, Mobley, Ratikainen, & Rosenqvist, 2013; Aronsen, Mobley, et al., 2013; Dreiss, Cote, Richard, Federici, & Clobert, 2010; Elmberg, 1991; Head et al., 2007; de Jong, Wacker, Amundsen, & Forsgren, 2009; Lu, Ma, Fan, & Yu, 2010; Wacker et al., 2013). Second, it is crucial to test mate competition and mating preference in the social environment in which the populations evolved and adapted, which requires using the OSR and density encountered by natural populations in the wild (Fromhage,

Elgar, & Schneider, 2005; Fromhage, McNamara, & Houston, 2008; Kokko & Jennions, 2008).

Here we aimed to test whether the set-ups widely used in mating success experiments in the model butterfly *Bicyclus anynana* promote sexual harassment of females by males and impede female mate choice, given that these set-ups probably differ from the natural conditions occurring in the field. This species is an excellent model species to address the effects of varying OSR and density on the expression of male–male competition and female mating preferences. Indeed, sex roles in *B. anynana* switch from conventional (with males competing for female access) to reversed sex roles across seasons (Prudic, Jeon, Cao, & Monteiro, 2011). This suggests considerable flexibility in the mating behaviour of both sexes, possibly caused by changes in OSR and/or density. In the wild, natural courtship in *B. anynana* involves a perch-and-chase strategy for mate location (Brakefield & Reitsma, 1991; Breuker & Brakefield, 2002). We lack a detailed description of male mate location strategies in the field, but both perch-and-chase and patrolling strategies are commonly observed in the laboratory at all experimental densities (Joron & Brakefield, 2003; Nieberding et al., 2008; Prudic et al., 2011; Robertson & Monteiro, 2005; this study). Perching males can be found locally at high densities, where they are frequently involved in male–male competition evidenced by circuit and chasing flights, such chases being interrupted by repeated alightings (Brakefield & Reitsma, 1991; Janowitz & Fischer, 2010; Joron & Brakefield, 2003). In the wild, the OSR is biased towards males and males can mate many times whereas females rarely mate more than once (Brakefield & Reitsma, 1991). There is limited available information regarding field sex ratios and densities for *B. anynana*. The only published report of field sex ratios of which we are aware comes from a 3-year survey in Malawi (Windig, Brakefield, Reitsma, & Wilson, 1994) and mentions a sex ratio of 62% of males based on daily capture in three bait traps. Our own field data from Uganda (Holveck, Gauthier, & Nieberding, 2013) provided an average sex ratio of 84% of males (range 57−100%, $N = 1063$ butterflies collected once a day in 7−29 banana bait traps in six sites of 0.25−6.02 ha for a total of 26 collection days). Regarding field density, on average 47 *Bicyclus* butterflies were caught per ha per sampling round (range 15−115) in Central Uganda, among which an average of 19 butterflies/ha may belong to *B. anynana* species (range 8−35) considering an even species diversity (pooled data from three sampling methods: transect walk-and-counts, hand netting for 20−30 min per transect and banana bait trapping over 2 consecutive days, Munyuli, 2012). Our own field data from Uganda (Holveck et al., 2013) produced a density of 42 ± 50 *B. anynana* butterflies/ha per sampling round (mean ± 1SD; range 1−158, $N = 1063$ butterflies). The field density values reported here should be treated with caution since they strongly depend on the sampling methods used to assess species richness (e.g. bait trapping is less than half as effective as capture by hand net; Munyuli, 2012), and were not estimated by capture−recapture experiments. These values nevertheless overlap estimates of *B. anynana* population size by capture−recapture experiments in Malawi (with banana bait trapping or capture on natural fruit falls for 2 months) where field density was 3−13 butterflies/ha, as retrieved from the study of Brakefield and Reitsma (1991) after correction by the relative density of *B. anynana* and *Bicyclus safitza*.

In the laboratory, numerous experimental studies of mating success have recently been performed for *B. anynana* (30 published studies; see Fig. 1). While the laboratory sex ratios (25−80% of males) fall within the range of field ones (62−84% of males; see above), most laboratory studies used a range of densities (0.0002−4 butterflies/dm^3, which can be roughly converted to two