FORUM

Estimating female morph frequencies and male mate preferences of polychromatic damselflies: a cautionary note

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In recent years, the adaptive significance of female-limited colour polymorphism in damselflies has been intensively studied (reviewed by Cordero & Andrés 1996). Polychromatic species have up to three female morphs, one androchrome (resembling a male in colour) and one or two gynochromes that are clearly different from males in body coloration and (sometimes) pattern (Cordero et al. 1998). Androchromes are such a perfect male mimic in some species that even odonatologists might confuse them with males (e.g. the picture of a male *Ischnura elegans* in Pecile 1984 is in fact an androchrome female). Robertson (1985) and Hinnekint (1987) proposed that males cannot distinguish these females from other males, and suggested that frequency- or density-dependent selection might be the main factors that contribute to the maintenance of this polymorphism.

Other authors have proposed that males are simply more interested in the most common female morph in their population (Fincke 1994; Van Gossum et al. 1999), and predicted that males should change their mate preferences according to morph frequency. In spite of this prediction, Forbes & Teather (1994) indicated that there were inconsistencies between studies, and remarked that conspecific males are not always attracted to the most common morph. Most previous studies have found that males are more attracted to gynochrome females which are usually the commonest morph. This has been done by presenting live tethered models to males in natural populations (see Table 1 for references) or by observing the interactions between free-living specimens (Sirot 1999). Nevertheless, when dead pinned models are presented, in most studies all morphs are highly attractive to mate-searching males (Table 1). These facts suggest that model behaviour is as important as coloration in male mate preferences (Cordero et al. 1998).

The recent paper by Van Gossum et al. (1999) on *I. elegans* reported that males were more attracted to the commonest morph in five populations. This conclusion was based on the finding of a positive correlation between the abundance of the most common female morph in the population and male choice for that morph. Nevertheless we think that their conclusions are premature, because they estimated female frequencies from very small samples. Our aim in this paper is twofold: (1) to determine the minimum sample size and sampling time needed to estimate female frequencies correctly and (2) to review critically the methodology and results of experiments designed to test male mate preferences in damselflies.

Methods

We reanalysed large samples of polychromatic damselflies obtained by Cordero and coworkers (Cordero 1992; Andrés 1998; Cordero & Egido 1998; Cordero et al. 1998; Cordero & Andrés 1999), including four populations of *Ischnura graellsii*, one of *I. elegans*, one of *I. pumilio* and one of *Ceriagrion tenellum* (studied over 2 years). All samples were obtained during mark–release–recapture studies and are based on ca. 1 month of field work per site. We calculated the frequency of androchrome females on a daily basis, as the proportion of androchromes to all females observed that day (reobservations of the same female on different days were also included). To estimate minimum sample size, we calculated the frequency of androchromes for increasing samples, from a minimum of 10 females to the complete sample of that population, in the same sequence as the females were found during field work in the population.

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Table 1. A review of studies of male preference in polychromatic damselflies

Species	Androchrome frequency (%), sample size, population and sampling period	Method to estimate male preference	Sexual response to androchomes and androchrome frequency (%) at test site	Sexual response to gynochromes (%)	References
Argia apicalis	48 (N=115), 47 days	Paired presentation. Dead models	35 (9/26) / 48	65 (17/26)	Bick & Bick 1965
Argia vivida	64–65 (N=78–448), Halcyon Hotsprings, 3 or 24 days; 48 (N=33), Albert Canyon, 4 days	Presentation of one morph at a time. Models pinned alive to end of stick	54 (13/24) / 65	56 (14/25)	Conrad & Pritchard 1989
Calopteryx splendens	10–15 (N=31–61), 19 days	Presentation of one morph at a time. Live tethered individuals	85 (N=78) / 10–15	80 (N=46)	De Marchi 1990
Ceriagrion tenellum	12 (N=657), As Graveiras, 51 days	Presentation of one morph at a time. Live tethered models; Presentation of one morph at a time. Dead models	63 (N=30) / 12; 60–73 (N=30) / 11–27	82 (N=79); 63–80 (N=60)	Andrés 1998
Coenagrion puella	20 (N=20), 1 day	Presentation of one morph at a time. Dead models	54 (97/178) / 20	78 (119/152)	Gorb 1998
Enallagma boreale	57–78 ($\bar{X}$=68%, N=485), 7 days	Paired presentations. Dead models	66 (3.3/5) / 68	32 (1.6/5)	Forbes 1994
Enallagma boreale	E. boreale: 65; E. ebrium: <5	Paired presentations. Dead models of E. boreale presented to male E. ebrium	16 (4/25) / 65	84 (21/25)	Forbes 1991
Enallagma ebrium	2.9–15 ($\bar{X}$=6.7, N=270, 26–36/day), 9 days	Paired presentations. Dead models	55 (67/122) / 6.7	45 (55/122)	Forbes & Teather 1994
Enallagma hageni	26 (N=39), 2 days; 26–36 (N=103–370), 18–33 days	Presentation of one morph at a time. Live tethered individuals	35 (63/178) / 26	67 (139/207)	Fincke 1994
Ischnura denticollis	18 (N=38), 25 days	Presentation of one morph at a time. Live tethered individuals	49 (17/35) / 18*	86 (31/36)	Córdoba Aguilar 1992; Córdoba Aguilar 1993
Ischnura elegans	51 (N=450), every 3 days from 8 Sep–10 Nov	Paired presentation of live females in small cages	32–61 (N=18–30) / 16–70	39–68 (N=18–30)	Van Gossum et al. 1999
Ischnura elegans	16–70 (N=10–17), 2 days per site	Presentation of one morph at a time. Dead models	68 (N=275) / 24	54 (N=250)	Gorb 1999
Ischnura elegans	24 (N=202), 3 days	Presentation of one morph at a time. Live tethered models; Presentation of one morph at a time. Dead models	33 (10/30) / 55; 83 (25/30) / 55	62 (37/60); 92 (55/60)	Cordero et al. 1998
Ischnura graellsii	14 (N=685), 36 days	Presentation of one morph at a time. Live tethered models	33 (9/27) / 14	89 (48/54)	Cordero 1989; Cordero 1990
Ischnura ramburii	61±3, SW pond; 42±5, ACF pond (N=221), several days from Oct 1997 to Sept 1998	Observation of interactions of free individuals	14 (3/22) / 61	68 (15/22)	Sirot 1999
Ischnura ramburii	31 (N=90), 2 days	Presentation of one morph at a time. Models pinned alive to end of stick	55 (11/20) / 31	75 (15/20)	Robertson 1985
Nehalennia irene	2.1–28.1 (N=15–156), 2 days per site	Presentation of one morph at a time. Models glued alive to end of stick	43 (9/21) / 22–30	79 (15/19)	Forbes et al. 1997; Forbes et al. 1995

In species with more than one gynochrome female, all were grouped. Sexual response was calculated excluding males that did not react to models.
In the case of A. apicalis (Bick & Bick 1965) we considered androchrome females with 'dark' or 'turquoise' thorax, because males showed the same coloration (see Dunkle 1990).
*A. Córdoba Aguilar, personal communication, 1993.

Results

In all populations studied, the frequency of andro-chromes changed greatly between days (Fig. 1), but only in the *I. elegans* population was a significant correlation found between androchrome frequency and date (Kendall's rank correlation coefficient: $\tau= -0.33$, $N=25$, $P<0.05$). The coefficient of variation of androchrome frequency was 18% for *I. elegans*, 26–73% for *I. graellsii*, 110% for *I. pumilio* and 61–76% for *C. tenellum*.

Sample sizes of fewer than 100 females will rarely be a good estimate of the true population frequency. It is only above this that the frequency tends to become independent of sample size (Fig. 2).

Male preference for female colour morphs has been studied in 12 Coenagrionidae and one Calopterygidae species (Table 1). Some studies have presented live tethered females to males and recorded whether males responded in a sexual or nonsexual way to the model. Other studies used immobile models (either dead or alive, paired or not) glued or pinned to the tip of a stem. Finally, a few followed male–female interactions in free-living specimens (Sirot 1999), or used small cages where males were allowed to choose between two females (Van Gossum et al. 1999). We have calculated the percentage of males that showed sexual interest to androchrome models, excluding males that did not respond (Fig. 3). This measure of male interest in androchromes does not correlate with the frequency of this morph in the population (all experiments: Spearman correlation: $r_s= -0.14$, $N=19$, $P=0.566$; experiments with live models (tethered, glued or pinned) presented one at a time: $r_s= -0.46$, $N=10$, $P=0.184$; all experiments with dead models: $r_s=0.14$, $N=7$, $P=0.758$).

Discussion

The adaptive significance of female colour poly-morphism in damselflies is still unclear. In most species so far studied males were clearly more attracted to gyno-chromes than to androchromes (Table 1; see also Cordero & Andrés 1996). This fact was interpreted as evidence for the male-mimic hypothesis (Robertson 1985; Hinnekint 1987), but it could also be the result of a male preference for the commonest morph, because in most populations studied so far androchromes were the minority morph (Fincke 1994). For instance, Forbes (1991) found that males of *Enallagma ebrium* were more attracted to gynochrome *E. boreale* females, the majority morph in the *E. ebrium* population, but the minority morph in *E. boreale*.

Johnson (1975) indicated that male *I. damula* and *I. demorsa* have an innate preference for either andro- or gynochrome females, but remarked that this preference could be broken down by maintaining males separated from females for 2–5 days. After this period, males also attempted mating with the less preferred morph. Male preference was not found in field studies that followed marked males during their lives. Considering males that mated twice, in all cases the observed frequency of different mating combinations was not significantly different from the expected given the relative frequency of female morphs in the population (*I. graellsii*: Cordero 1992; *E. boreale* and *E. hageni*: Fincke 1994; *I. elegans*: Cordero et al. 1998; *C. tenellum*: Andrés 1998). Van Gossum et al. (1999) presented evidence for male preference towards the commonest morph in the damselfly *I. elegans*. They studied five populations and did binary choice experiments in small cages, and concluded that males were choosing the commonest morph in each population. We think that the relationship between male preference and androchrome frequency has not been adequately shown, because androchrome frequencies were estimated from samples of only 10–17 females per population. Take for instance the data presented in Fig. 1: in the *I. graellsii* population studied in 1987, androchromes were 46% in one sample of 31 females but only 10% in a sample of 75 females taken only 3 days later. Similar examples can be seen in some of the other populations. The correlation between male preference and female frequency found by Van Gossum et al. (1999) is in fact due to the effect of only one point: the Neerhelst population, the only one where males showed a significant preference for one morph, not surprisingly the gynochrome. But the androchrome frequency of that population was estimated from one female out of a total of 13! Had their sample included only one more androchrome (2 out of 14) the correlation would disappear (Pearson correlation on arcsine transformed data: $r=0.62$, $P=0.133$, one-tailed). The reason for the small sample size was the low density of their populations (H. Van Gossum, personal communication).

Our *I. pumilio* population was also at a low density and, as Fig. 1 shows, androchromes were 10–20% from 31 August to 3 September when we observed 69 females but 0% from 4 to 8 September with 17 females, the maximum sample size of Van Gossum et al. (1999). This raises the question of which time period should be used to estimate female frequencies. A working solution of this dilemma could be to estimate morph frequencies at the same time as mate selection is being studied.

Fincke (1994) indicated that, if morph frequency is estimated during a short period, there is a risk of making erroneous conclusions if androchrome frequency shows seasonal changes, as occurred with her *E. boreale* and our *I. elegans* populations. In most populations these seasonal changes have not been found, but daily oscillations in androchrome frequencies are conspicuous. Figure 1 clearly shows that androchrome frequencies estimated from about 100 females or more (as in *I. graellsii*, 1996) are less variable than frequencies estimated from smaller samples. We therefore suggest that future work on this topic should count about 100 females, and never fewer than 50. Ideally, populations should be sampled over several days.

A second problem to test for frequency- and density-dependent male preference is the variety of experimental procedures used by different researchers (Table 1). Fincke (1994) first proposed that there was a positive relation-ship between androchrome frequency and male sexual interest in androchromes in a survey of the literature. Nevertheless, Cordero & Andrés (1996) indicated that this relationship did not hold if experiments with live and
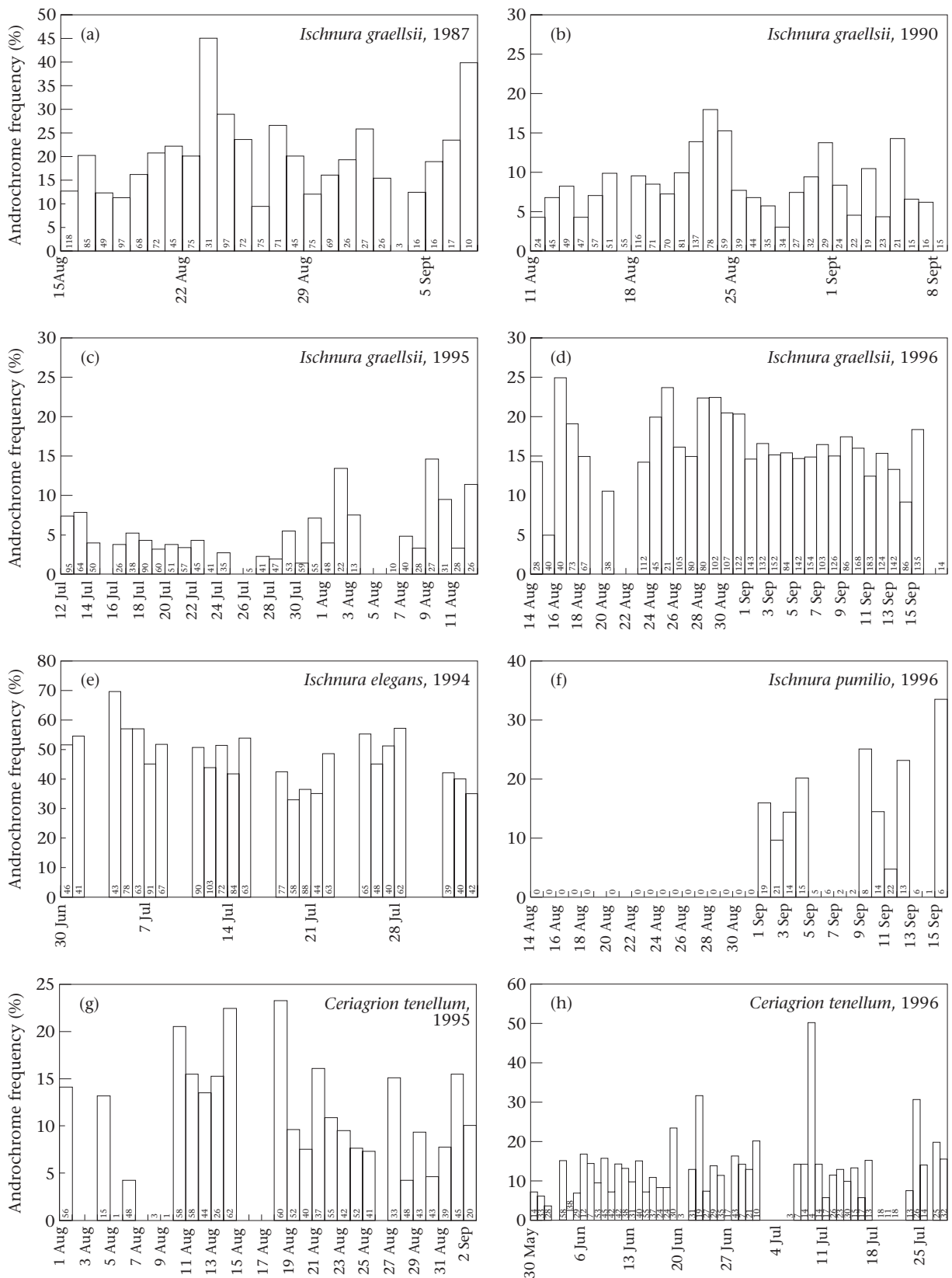
Figure 1. Daily variation in androchrome frequency in polychromatic damselflies. Numbers above the *X* axis indicate sample size. Data from Cordero 1992; Cordero et al. 1998; Andrés 1998; Cordero & Egido 1998 and Cordero & Andrés 1999.
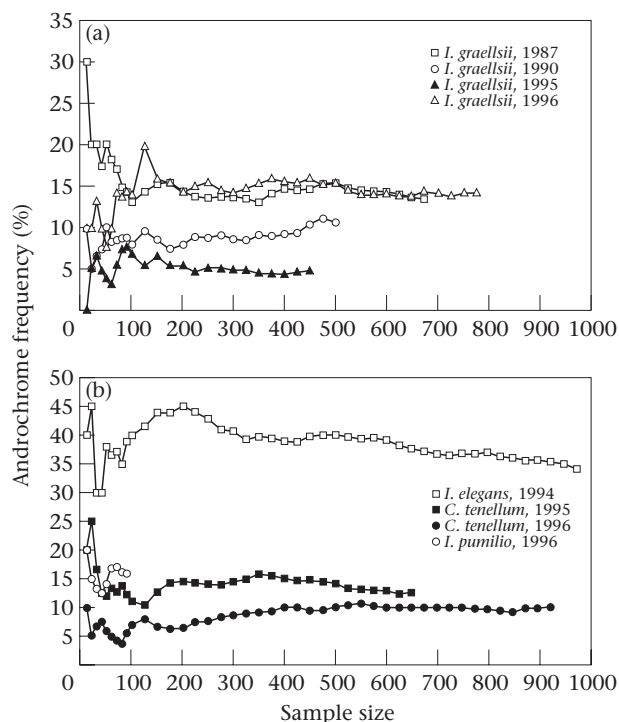
Figure 2. The relationship between sample size (increasing by 10 females until a sample size of 100 and 25 females thereafter) and androchrome frequency in field studies of polychromatic damselflies.
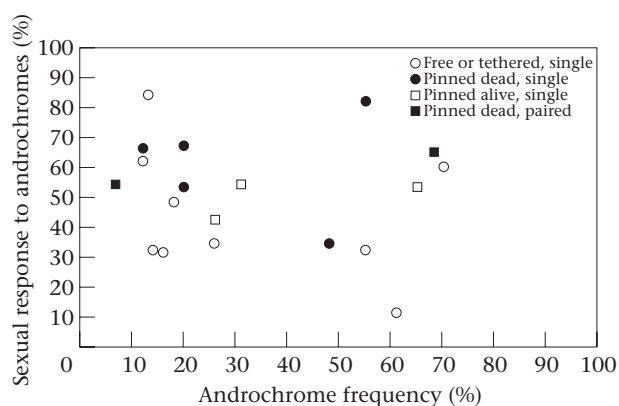

Figure 3. The relationship between androchrome frequency and sexual response to androchromes in studies of model presentation to males of polychromatic damselflies.

dead models were separated. Male damselflies mate rather indiscriminately, even forming male–male tandems and triple connections (Corbet 1999). They are attracted to any perched damselfly. Therefore, if we want to test which morph elicits more mating attempts, we cannot use dead models, because they cannot respond to the approaching male. Van Gossum et al. (1999) reported a significant relationship between androchrome frequency and male preference in a comparative review of the literature, including their observations of binary choice in small cages, paired presentations of dead models and data from field observations. Nevertheless, we found no

relationship between these variables (Fig. 3). This discrepancy could be due to a slightly different way of defining male preference: they calculated male preference as the number of males that attempted mating with androchromes divided by the number of males that attempted mating with all females. We calculated male preference as the number of males that attempted mating with androchromes divided by the number of males that were tested with androchrome models. Van Gossum et al (1999) assumed that males will choose only one female morph and their estimate of male preference for androchromes added to male preference for gynochromes will therefore add up to 100%. Our estimates are not additive: males can show 80% preference for androchromes and also 80% preference for gynochromes. This allows for males that are indiscriminate and try to mate with all females, which is common in field trials of male preference (Table 1).

If all studies on mating frequency in polychromatic damselflies are taken into account, in most cases the androchrome frequency in mating pairs does not deviate significantly from the androchrome frequency in the population and, comparing between species, there is a strong correlation between the two variables ($r=0.92$, $N=17$, $P<0.001$; data obtained from references in Table 1). Nevertheless, because the majority of females in tandem or copula are already marked, this is a clear case of autocorrelation; the androchrome mating frequency is estimated from a sample almost identical to the population sample. This might explain why males seem to mate randomly in field studies, but nevertheless show clear preferences when tested. We need independent estimates of female morph frequencies and mating frequencies.

Van Gossum et al. (1999) excluded from their comparison all experiments done with only one model per trial because 'besides male choice, willingness to mate is also a factor'. This could be a problem only if willingness to mate is related to female phenotype: that is, if males do not try to mate with all females with the same probability. Males could try to mate only if the preferred morph is found and ignore other mating opportunities. Nevertheless, given that the sex ratio is heavily male biased in mature populations, males should not be choosy. Furthermore, it is very unlikely that males have the opportunity to choose between females in the field, because only under very high densities will they be likely to find two females simultaneously (see also Silberglied 1984 for a similar discussion in butterflies).

Comparisons between female morphs presented at the same time (simultaneous tests) provide the more sensitive measure of male preference, while presentation of each female morph in turn provides the more rigorous test. On the one hand, simultaneous presentation tests tend to be highly unnatural and males may become trapped by their first choice. On the other hand, successive (or single trial) presentations may be insensitive if males respond at the maximum possible level to each female when they cannot make simultaneous comparison between female morphs (see Martin & Bateson 1986). We therefore suggest that future work on mate choice in damselflies should include both simultaneous and sequential (or

single trial) presentations, although presenting only one morph at a time is the most natural experiment. The approach used by Sirot (1999) and Cook et al. (1994) of observing male–female interactions among undisturbed specimens is even better, but unfortunately it is much too time consuming.

If all studies done with live models presented one at a time are considered, there is no relationship between male preference and androchrome frequency (Fig. 3). In six of the studies presented in Fig. 3 androchromes were the commoner morph, but there was no increase in male response to them, and there is even a case were androchromes made up 10% of the population but males showed 85% positive responses to this morph (De Marchi 1990). In conclusion, an accurate test of the idea that males really do prefer the commonest female morph in polychromatic damselflies is still lacking.

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