No association between brain size and male sexual behavior in the guppy

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Abstract Animal behavior is remarkably variable at all taxonomic levels. Over the last decades, research on animal behavior has focused on understanding ultimate processes. Yet, it has progressively become more evident that to fully understand behavioral variation, ultimate explanations need to be complemented with proximate ones. In particular, the mechanisms generating variation in sexual behavior remain an open question. Variation in aspects of brain morphology has been suggested as a plausible mechanism underlying this variation. However, our knowledge of this potential association is based almost exclusively on comparative analyses. Experimental studies are needed to establish causality and bridge the gap between micro- and macroevolutionary mechanisms concerning the link between brain and sexual behavior. We used male guppies that had been artificially selected for large or small relative brain size to study this association. We paired males with females and scored the full known set of male and female sexual behaviors described in guppies. We found several previously demonstrated associations between male traits, male behavior and female behavior. Females responded more strongly towards males that courted more and males with more orange coloration. Also, larger males and males with less conspicuous coloration attempted more coerced copulations. However, courting, frequency of coerced copulation attempts, total intensity of sexual behavior, and female response did not differ between large- and small-brained males. Our data suggest that relative brain size is an unlikely mechanism underlying variation in sexual behavior of the male guppy. We discuss these findings in the context of the conditions under which relative brain size might affect male sexual behavior [Current Zoology 61 (2): 265–273, 2015].

Keywords Brain size, Brain morphology, Sexual behavior, Guppy, Artificial selection, Behavioral mechanisms

Behavioral variation is ubiquitous at all biological levels and what underlies this variation is a long-standing question in biology (Darwin, 1859; Tinbergen; 1963, Alcock; 2013). Behavioral ecology has been incredibly successful in elucidating the ultimate processes that generate this variation (Milinski, 2014). Yet, to fully understand behavior, it has become evident that more information is needed on the mechanistic background of behavioral variation (Bateson and Laland, 2013).

The brain, as the central unit controlling behavior, is a logical focus of interest when investigating the mechanistic basis of behavioral variation. Indeed, the interplay between variation in brain morphology and behavioral patterns has received considerable attention (Dunbar, 1998; Allman, 2000; Northcutt, 2002; Striedter, 2005; Hofman and Falk, 2012). For instance, relative brain size (i.e. brain size controlled for body size), a relatively coarse attribute of brain morphology, correlates with a wide spectrum of behavioral and behavior-related traits, including parental care (Gittleman, 1994; Gonzalez-Voyer et al., 2009a), mating system (Pollen et al., 2007), diet (Iwaniuk and Nelson, 2001; Gonzalez-Voyer et al., 2009b), social group size (Dunbar, 1998; Lindenfors, 2005), and migratory behavior (Sol et al., 2005). On a finer scale, variation in brain structure is also linked to behavioral variation as exemplified by the relatively larger hippocampus in food caching species (Volman et al., 1997). These comparative studies provide insights into phylogenetic patterns and suggest ultimate explanations for the link between brain morphology and behavior. To fully understand cause and effect, such macroevolutionary analyses should ideally also be complemented by experimental analyses at the within-species level (Kotrschal et al., 2013a, Kolm, 2014).

Sexual behaviors have intrigued scientists and laymen alike, even before Darwin (1871) developed the first theories of sexual selection. However, despite a long-standing tradition in sexual selection research (Anderson, 1994; Eberhard, 1996; Ryan, 1998; Ritchie, 2007),
how variation in sexual traits is generated and maintained in the light of strong sexual selection remains a major question in evolutionary biology (Arnqvist and Rowe, 2005). Furthermore, variation in aspects of brain morphology has been suggested as a factor underlying variation in sexual behavior at the individual and species level (Jacobs, 1996; Balaban, 1997; Boogert et al., 2011; Kotrschal et al., 2012). For instance, brain size correlates positively with sexual traits across several taxa, such as bower complexity in bowerbirds (Madden, 2001), intensity of sexual selection in birds (Garamszegi et al., 2005), and mating success determined by social ranking in primates (Pawlowski et al., 1998). Brain size has further been found to correlate negatively with the degree of polyandry in bats (Pitnick et al., 2006; but see Lemaître et al., 2009). In addition, a negative correlation has been found between brain structure, telencephalon size, and the degree of polygamy in cichlid fishes (Pollen et al., 2007).

Previous studies demonstrate that the link between brain morphology and cognitive ability is likely to mediate behavioral variation in contexts such as spatial behavior (Jacobs et al., 1990), innovation (Lefebvre et al., 1997; Reader and Laland, 2002), and tool use (Lefebvre et al., 2002; Tebbich and Bshary, 2004), although such comparative studies need to be interpreted with caution (Chittka and Niven, 2009). Recent theory concerning the mechanistic background of sexual behavior suggests that it is the positive association between cognitive abilities and brain size that affects sexual behavior (Miller, 2000, see review by Boogert et al., 2011). Variation in cognitive ability is thought to have a strong impact on individual fitness during mate choice for both the signaler and the receiver of sexual signals and displays (Andersson, 1994; Shettleworth, 2010; Kotrschal et al., 2012). The most straight-forward association between cognitive ability and sexual behavior can be observed when courtship behavior is cognitively demanding itself (Boogert et al., 2011). Some traits that are under sexual selection consist of complex displays which involve long learning periods and much practice before they are fully developed, as is the case with song-learning ability in certain birds (Novicki et al., 1998; Nowicki et al., 2002). Furthermore, complexity of sexual behavior has been suggested to vary depending on brain morphology, such as the coevolution of brain size and bower complexity across bowerbird species (Miller, 2000; Keagy, 2009) and brain size and acrobatic courtship displays in manakins (Lindsay et al., 2015). In view of these findings, to address the mechanistic basis of sexual behavior it is important to consider sexual behavior in the light of brain morphology variation, particularly in species with a known association between brain morphology and cognitive ability.

One experimental approach to study the link between brain morphology, cognitive ability and behavioral traits is manipulation of brain morphology via artificial selection. Indeed, artificial selection has proven to be a powerful tool in brain evolution research (Mery and Kawecki, 2005; Kotrschal et al., 2013a). Work on the recently developed brain size selection lines of guppies Poecilia reticulata, artificially selected for large or small relative brain size (Kotrschal et al., 2013a), has shown considerable differences in behavior between the lines (Kotrschal et al., 2014a), including better performance in cognitive tasks by individuals with larger brains (Kotrschal et al., 2013a, b; Kotrschal et al., 2014b). These brain size selection lines now offer us the opportunity to test experimentally how known differences in brain size and cognitive ability may relate to differences in sexual behavior.

Here we experimentally test the hypothesis that brain size and cognitive ability affect male courtship and copulatory behavior in replicated lines of male guppies artificially selected for large and small relative brain size. The guppy is a model organism for sexual selection research (Kodric-Brown, 1993; Houde, 1997; Brooks and Endler, 2001; Kodric-Brown and Nicoletto, 2001; Godin et al., 2005) and the male sexual behaviors have been described in great detail (see Houde, 1997). Briefly, prior to copulation, males follow and circle around the females to display their sexual ornaments, such as their body coloration. In addition, males swing their gonopodium (the intromittent organ of this internally fertilizing species) in front of the females. In order to complete copulation, males display a characteristic s-shaped (sigmoid) display in front of the female thereby seeking her collaboration. Frequently, male guppies also exhibit a complementary ‘sneak’ copulation behavior where they perform coerced copulations without female consent. Since successful courtship in the guppy requires carefully coordinated movements and awareness of behavioral cues from both male competitors and courted females (Jirotkul, 1999; Head and Brooks, 2006), we hypothesize that this places demands on multiple cognitive traits such as perception, attention and/or decision-making. Moreover, when guppy males attempt coerced copulations, they often sneak up on females from behind and this requires an ability to respond to and synchronize with female movement patterns, which has been sug-
gested to be cognitively challenging (Houde, 1997; Fraser, 2014). In our study, we paired large- and small-brained males with non-selected females and simultaneously examined male sexual behavior and female response behavior. If relative brain size affects sexual behavior, we predict differences between large- and small-brained males in sexual behaviors such as courting and/or attempting coerced copulations with the female.

1 Materials and Methods

1.1 Study system

We studied the association between brain morphology and reproductive behavior in male guppies from laboratory populations previously artificially selected for small or large relative brain size (Kotrschal et al., 2013a). Briefly, two relative brain size treatments (three replicates for large- and three replicates for small-brained, six populations in total) were performed on animals of a large outbred laboratory population of Trinidadian guppies (Kotrschal et al., 2013a). To select for relative brain size, brain weight data corrected for body size were collected from 225 pairs (75 per replicate) after they had produced two clutches each. Brains from euthanized parents were removed under a stereomicroscope and weighed. Offspring from the top and bottom 20% were then used to set up the large- and small-brain selection lines. To avoid inbreeding, full-siblings were never mated. See Kotrschal et al. (2013a) for full details on the selection experiment. These selections showed a 9% difference in relative brain size in the second generation (Kotrschal et al., 2013a), and an 11% difference in the third generation (Kotrschal et al., 2015). Furthermore, measurements performed on a subsample of individuals from the fourth generation showed a 13.6% difference in relative brain size (Kotrschal et al., 2014a).

For the present experiment, we used a total of 60 males from the fourth generation, 10 individuals from each of the three up- and down-selected lines. All fish were removed from their parental tanks after birth and males were isolated from females before they were sexually mature. Once sexually mature, the fish were transferred to single 3-L tanks containing 2 cm of gravel with continuously aerated water. We allowed visual contact between the tanks. The laboratory was maintained at 26°C with a 12:12 light:dark schedule, which resulted in 25°C water temperature. Fish were fed a diet of flake food six days per week.

1.2 Sexual behavior and physical traits

To study the association between brain size and male sexual behavior we measured a set of sexual behaviors in males that were individually placed in a tank together with a sexually mature female guppy from a non-selected laboratory population. The quantification of behavior took place in a 19 × 28 × 10 cm observation tank with fine gravel. Water was changed every day and temperature was held constant at 25°C. We placed the female and male guppy in the observation tank simultaneously. Both individuals were fed in their home tanks one hour prior to the trial. Behavior of both animals was recorded for 25 min from the top view using a Logitech HD webcam and from the side view using a Sony HDR-SR11 video camera. Six trials per day during 10 consecutive days were performed between 08:00 am and 12:00 pm. In total 30 males of each brain size selection line (10 per replicate) were tested. We tested one individual of each replicate and brain size combination per day, with the order randomized on each day. After the behavioral trials, we collected measurements of physical attributes of all participating individuals. In females, we measured standard length (body length, from the tip of the snout to the end of the caudal peduncle). For males, we took photographs and performed image analyses with ImageJ software v. 1.44 (Schneider et al., 2012) to quantify their standard length, tail size and orange coloration (all traits were corrected for body size).

We quantified male sexual behaviors using Jwatcher software v. 1.0. (Blumstein and Daniel, 2007). Quantification was performed blind to the treatment since only running numbers identified individuals. We used videos recorded from the side view to measure the characteristic male guppy courting behaviors (for detailed description see Baerends et al., 1955; Liley, 1966): the frequency of forward movements of the gonopodium (‘gonopodial swings’); frequency of gonopodium thrusts towards the female genital pore from a behind position (‘coerced copulation attempts’); and frequency and average duration of the characteristic s-shaped quivering movement performed in front of the female (‘frequency of sigmoid display’ and ‘average duration of sigmoid display’). We also used videos from the top view to measure the total and average time that males actively chased the female to within the distance of an average female body size (‘time following’ and ‘average following bout’). In addition, we normalized every male sexual behavior (not including latencies) between 0 and 1 using maximum and minimum values. We then obtained a score for the total intensity of male sexual behavior by summing up these values for every individual.
Furthermore, we used top-view videos to assess female response to male courtship. For this, we measured the number of times that females oriented their bodies towards the male (‘orientation’), the number of times a female slowly approached the male after sigmoid displays (‘gliding response’), and the total time a female spent escaping from males (‘time escaping’). Orientation and gliding response have been shown to be correlated with the probability of accepting a given male, while the time escaping has been shown to correlate with rejection (Liley, 1966; Houde, 1987; Nicoletto, 1993). In addition, a score for the total female response was obtained combining normalized values of the frequency of orientations and gliding responses.

1.3 Statistical analyses

To evaluate how male and female physical traits relate to male sexual behaviors and female responses, we studied their correlations based on the combined data of small- and large-brained individuals. We had clear predictions concerning these correlations based on previous literature (Farr, 1980; Houde, 1997; Kodric-Brown and Nicoletto, 2001; Karino and Shinjo, 2004). Therefore, we kept the critical value (α) at 0.05 and did not correct for multiple testing (Nakagawa, 2004).

To test for potential differences in sexual behavior between the relative brain size selected lines, we used a Linear Mixed-Effects Model (LMM) for every male sexual behavior scored as the dependent variable. Full models included brain size as a fixed effect, a random intercept and slope for brain size for each replicate and time of the day, female size and male physical attributes (standard length and orange coloration) as covariates. In addition, full models included the full interaction between brain size and male/female attributes (lme4 syntax: y ~ brain.size * female.size * male.standard-length * male.orangecoloration + time of day + (brain. size | replicate)). We performed a stepwise backward model selection based on Akaike Information Criteria (AIC), only varying the fixed effects structure (Zuur et al., 2009). Even though the interaction in the full model was non-significant for all of the male sexual behaviors scored, removing it from the model resulted in a distinct increase of the AIC value. In addition, we observed no changes in the outcome of simpler models. Also, using female behavior data we tested whether females responded differently to individuals of large and small relative brain size. We again used brain size as a fixed effect and the random intercept and slope for brain size for each replicate. In this case the model with the lowest AIC did not include any covariates.

We found multiple significant correlations between the male sexual behaviors. Therefore, to maximize the power of the analysis and reduce the number of inferential tests, we performed a principal component analysis on the correlation matrix of the male sexual behaviors (Quinn and Keough, 2002). We excluded latencies in the PCA since, although they were correlated with the frequencies, these observed latency correlations did not differ from values obtained by simulating expected latency based on frequency data (sigmoid displays: ρ = -0.62, P = 0.43; coerced copulation attempt: ρ = -0.68, P = 0.64; gonopodial swings: ρ = -0.43, P = 0.55). Two components presented an eigenvalue > 1. The first principal component, which explained 41% of the variation, was characterized mainly by three aspects: the time that males spent following females at a close distance, the average duration of each following bout and the frequency of coerced copulation attempts. The second principal component, explaining 28% of the variation, was characterized mainly by the frequency of gonopodial swings and the frequency and average duration of sigmoid displays (Fig. 1A). We then performed a LMM using these components. We used an analogous model to the one described above to test for potential differences in sexual behavior between males with different brain sizes.

Model diagnostics showed that residual distributions were roughly normal with no signs of heteroscedasticity. Note that ten individuals were removed from the analyses because they did not perform sexual behavior during trials (5 from large-, 5 from small-brained lines). Nevertheless, we performed analogous analyses of the data including non-performing individuals and the results remained qualitatively identical. The final sample size used was 50 individuals, 25 from large-brained lines and 25 from small-brained lines. We performed all analyses in R v.3.1.1 (R Core Team, 2013) and used lme4 package version 1.1-7 (Bates et al., 2014) for LMM analyses.

2 Results

Several sexual behaviors measured in large- and small-brained male guppies correlated with their physical traits. Males with a lower proportion of orange coloration spent significantly more time chasing the female (r = -0.319, df = 48, P = 0.023), and showed a tendency to perform a higher number of coerced copulation attempts, although not significantly so (r = -0.236, df = 48, P = 0.098). In addition, larger males made a significantly higher number of coerced copulation at
Fig. 1  Principal component analysis of sexual behaviors of male guppies Poecilia reticulata, selected for large or small relative brain size, when presented to a female

Negative values of PC1 describe higher values of coerced copulation-related sexual behaviors while positive values of PC2 describe higher values of display-related sexual behaviors, as indicated by the direction of the arrows for specific behavioral measures. Larger symbols represent centroid values for small- and large-brained males. No difference is observed in the set of sexual behaviors between small- (circles) and large-brained (squares) males (A), or in the estimated marginal means and confidence intervals of a Linear Mixed Model (LMM) for values retrieved from the first component (B) and the second component (C) with brain size as a fixed effect, replicate as a random effect and time of day, female size and male physical traits as covariates.

We detected several of the expected patterns concerning the association between male physical traits and sexual behavior. However, we found no association between brain size and male sexual behavior. Artificial selection for relative brain size did not generate any discernible effects in any of the independent sexual behaviors studied, nor in the total intensity of sexual behavior. We also used a principal component analysis to group the courting- and coerced copulation-related behaviors, but we failed to detect differences in behavior between large- and small-brained males also with this method.

3 Discussion

We detected several of the expected patterns concerning the association between male physical traits and sexual behavior. However, we found no association between brain size and male sexual behavior. Artificial selection for relative brain size did not generate any discernible effects in any of the independent sexual behaviors studied, nor in the total intensity of sexual behavior. We also used a principal component analysis to group the courting- and coerced copulation-related behaviors, but we failed to detect differences in behavior between large- and small-brained males also with this method.

Is the lack of evidence for differences between large- and small-brained individuals an indication of no association between brain size and sexual behavior in the male guppy, or were the methods used inadequate to uncover such differences? Our design was powerful enough to pick up previously demonstrated associations between male traits and male and female behavior. For instance, we found that female response was correlated with the average duration of sigmoid displays (Farr, 1980), and the amount of orange coloration in males...
Furthermore, less conspicuous males tended to attempt coerced copulations to a higher extent (Houde, 1997), and larger males performed more coerced copulation attempts (Magellan et al., 2005). Hence, we argue that had there been a strong association between brain size and male sexual behavior, we should have been able to detect it. Our data therefore do not support the hypothesis that brain size affects male sexual behavior for the suite of behaviors studied in this set-up where males were not affected by competitive interactions with other males. This is somewhat surprising since previous comparative studies have flagged up a potential link between brain morphology and sexual behavior in different taxa, such as birds (Garamszegi et al., 2005; Madden, 2001; Lindsay et al., 2015), primates (Pawlowski et al., 1998), and bats (Pitnick et al., 2006). These examples differ from the present study in that they analyzed patterns across rather than within species. Our study offers the first experimental test on the relation between relative brain size and direct quantification of sexual behavior and, perhaps surprisingly, no association can be derived from our data. Variation in sexual behaviors in male guppies is generally very large (Rodd and Sokolowski, 1995, Magurran, 2005), and our results match these previous results (Table 1). However, our data suggest that this variation is not associated with brain size despite a difference of over 10% in relative brain size of the selection lines.

The set of sexual behaviors studied here might not require high cognitive abilities in male guppies. For example, male guppies appear to utilize the sexual behaviors we investigated without the need for learning, although at varying rates depending on several environmental factors (Houde, 1997). In contrast, performance of specific sexual behaviors in other species may require higher skills in cognitive traits such as greater memory and/or learning, as is the case with male sedge warbler Acrocephalus schoenobaenus songs (Airey et al., 2000) or bower-building to attract females in bowerbird species (Madden, 2001). When considered alongside our results, these examples suggest that brain morphology might affect sexual behaviors only when higher cognitive skills are necessary for the development of a more complex display. Our setup did not allow us to account for the role that past feedback and learning may have played in the sexual behavior of male guppies selected for relative brain size. Another explanation is that if brain morphology affects male sexual behavior in

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Number of gonopodial swings</th>
<th>Number of coerced copulation attempts</th>
<th>Time spent following the female</th>
<th>Average duration of sigmoid display</th>
<th>Latency to first behavior</th>
<th>Latency to first coerced copulation attempt</th>
<th>Latency to first sigmoid display</th>
<th>Normalized total male behavior</th>
<th>PCA component 1</th>
<th>PCA component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Test</td>
<td>Large brain size</td>
<td>Mean ± 2 SD (n)</td>
<td>40.15 ± 13.6 (25)</td>
<td>15.03 ± 15.26 (25)</td>
<td>23.25 ± 11.95 (25)</td>
<td>511.95 ± 339.99 (25)</td>
<td>3.24 ± 1.02 (25)</td>
<td>1.69 ± 0.63 (25)</td>
<td>12 ± 5 (25)</td>
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<td></td>
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<td>Difference of means</td>
<td></td>
<td>9.62 ± 7.62</td>
<td>2.50 ± 1.94</td>
<td>3.63 ± 0.18</td>
<td>65.95 ± 38.25</td>
<td>2.17 ± 1.02</td>
<td>0.56 ± 0.63</td>
<td>0.68 ± 0.56</td>
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<tr>
<td></td>
<td></td>
<td>Lower CI</td>
<td></td>
<td>5.42 ± 4.89</td>
<td>1.22 ± 1.22</td>
<td>1.35 ± 0.13</td>
<td>37.73 ± 20.73</td>
<td>1.03 ± 0.53</td>
<td>0.28 ± 0.53</td>
<td>0.30 ± 0.43</td>
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<tr>
<td></td>
<td></td>
<td>Upper CI</td>
<td></td>
<td>13.84 ± 10.75</td>
<td>3.74 ± 2.74</td>
<td>5.00 ± 0.35</td>
<td>103.76 ± 50.67</td>
<td>3.34 ± 1.83</td>
<td>1.84 ± 0.53</td>
<td>1.96 ± 0.43</td>
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<tr>
<td></td>
<td></td>
<td>p-value</td>
<td></td>
<td>0.950</td>
<td>0.382</td>
<td>0.612</td>
<td>0.226</td>
<td>0.681</td>
<td>0.902</td>
<td>0.775</td>
</tr>
</tbody>
</table>

Table 1 Differences in sexual behaviors between male guppies Poecilia reticulata, artificially selected for large and small relative brain size, when presented to a female
the guppy, variation may instead be driven by fine-scale differences in the relative size of brain subregions as has been suggested to occur in other species. For instance, Day et al. (2005) showed that not whole brain size, but cerebellum size, was associated with the differences inbower complexity across bowerbird species. In addition, a recent study at the microevolutionary level showed that individuals from a natural population of brown trout *Salmo trutta* presented differences in various aspects of brain structure depending on their mating tactic (Kolm et al., 2009). Therefore, it would be of great interest to study the association between brain substructures and sexual behaviors via artificial selection on morphological characteristics of separate brain regions.

As mentioned above, previous work on the artificially selected relative brain size lines in guppies found an association between brain size and cognitive ability (Kotrschal et al., 2013a, b; Kotrschal et al., 2014a). These studies showed that large-brained females and males performed better in cognitive tests designed to capture the specific ecology of the different sexes. Combining these results with the ones obtained in our present study, we suggest that relative brain size in this species mainly constrains aspects of cognition that are unrelated to male sexual display. There are some similarities between our findings and a recent study on bowerbirds that failed to find any association between cognitive ability and mating success (Isden et al., 2013). Interestingly though, Shohet and Watt (2009) found that female guppies preferred males that performed better in a learning task where males went through a maze to find a food item. Thus, male cognitive ability may be important in mate choice in the guppy, but mostly for behavior relating to ecological contexts other than courtship.

To conclude, we did not find support for a predicted association between relative brain size and male sexual behavior. Instead, we found high variability in the set of sexual behaviors that male guppies perform. However, further investigation is required before we can completely dismiss any link between brain size and male sexual behavior in this species. For instance, we investigated the behavior of pairs in a single interaction in the present study. The effect of relative brain size on sexual behaviors might be more evident in situations permitting social interactions between multiple individuals, allowing for male-male competition (Price and Rodd, 2006), or after multiple encounters with females in which learning may be involved (Jordan and Brooks, 2012). Hence, future studies should also take into account complex social interactions, such as dominance and aggression and/or the role of past experiences and repeated displays. Moreover, future studies should also investigate female behavior in these brain size selected lines in more detail before we can fully understand the effect of brain size on sexual behavior in both sexes.

**Acknowledgments** We thank Tim Fawcett and three anonymous reviewers for valuable comments on an earlier version of the manuscript. N. K. was funded by the Swedish Research Council. A.K. was funded by the Austrian Science Fund (J 3304-B24). The experiment was performed in accordance with the ethical regulations of Stockholm University and Swedish law.

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