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Distinguishing animal personality and innovation in the guppy - the effect of sex and body size

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Abstract

How individuals within the same population show consistent differences in different behaviours, i.e. animal personalities, and how these correlate into a wider behavioural syndrome has become a hot topic in behavioural research the last 20 years. Lately it has also been suggested that innovative behaviours should be considered as an aspect of animal personalities, since it has become clear that individuals differ in their innovative rate. In this study, guppies (*Poecilia reticulata*) were tested in four behavioural assays (exploration of an open area and a maze, and investigation of a novel object and a novel food). The individual variation and the co-variance between the measured variables, i.e. syndrome structure, was examined primarily by comparing seven *a priori* hypotheses using structural equation modelling (SEM). The best model was one that separated variables relating to activity from rates of exploration/investigation (i.e. rather than alternative divisions based on spatial vs. non-spatial or personality vs. innovation variables, etc.). No clear differences in syndrome structure were found when comparing the three different populations. However, there was some effect of individual body size on syndrome structure, because smaller females (but not males) were shown to be more active. Thus, syndrome structure here seems to be driven by individual, and possible sex, differences in developmental state, since size and age are highly correlated in fish. The role of such a syndrome is discussed in the context of guppy life histories, where the reproductive success of females, but not males, is size dependent.

Sammendrag

Hvordan individ i samme populasjon viser konsekvente forskjeller i adferd, kalt 'animal personalities', og hvordan disse adferdene korrelerer og danner adferdssyndrom har blitt et viktig tema innen adferdsøkologi de siste 20 årene. Det har blitt antydnet at innovativ adferd også skal inkluderes som en del av 'animal personalities', siden det er observer individuelle forskjeller i innovative rate hos flere arter. Innovativ adferd kan derfor være en del av et større adferdssyndrom hvor mer aktive og utforskende individ også er mer innovative. I denne studien blir guppier (*Poecilia reticulata*) observert i fire ulike adferdstester (utforskning av et nytt område, labyrint ukjent objekt samt ukjent mat), hvorpå kovariansene mellom de ulike adferdsvariablene – syndromstrukturen – ble undersøkt ved å sammenligne syv *a priori* hypoteser ved hjelp av structural equation modelling (SEM). Den beste modellen separerte adferdsvariablene relatert til aktivitet fra de relatert til utforskning (og ikke inn i 'animal personalities' og innovasjon eller romlige og ikke-romlige variabler). Det ble ikke funnet noen tydelig forskjell i syndromstruktur mellom de tre ulike populasjonene. Det ble derimot funnet at kroppsstørrelse hadde en effekt, hvor små individ var mer aktive enn store individ. Det kan derfor være at syndromstrukturen i dette systemet drives av guppyenes individuelle utviklingsstadium, siden størrelse korrelerer svært høyt med alder. Fra univariate tester ble effekten av størrelse kun funnet hos hunner, noe som kan komme av at størrelse påvirker fekunditet i mye større grad hos hunner enn hos hanner.

Table of content

Acknowledgement.....	I
Abstract	II
Sammendrag	III
Table of content	V
Introduction.....	1
Animal personality.....	1
Behavioural syndromes.....	3
Innovation	4
Linking Personality and Innovation	5
Methods	7
Study species	7
Experimental setup and video analysis	7
Exploration	8
Maze	9
Novel object	9
Novel food	10
Statistics	11
Repeatability and habituation.....	12
ANCOVA.....	13
Correlations between variables	13
Structural equation modelling.....	13
Results	15
Discussion	30
Conclusion	36
References.....	37
Appendix A	i
Appendix B	iii
Appendix C.....	iv

Introduction

Animal personality

Studies of human personality have been carried out by psychologists since the early twentieth century, but animal personalities have only received attention from behavioural ecologists for the last two decades (Freeman and Gosling 2006, Reale et al. 2007). It has long been known that animals show different degree of specific 'coping' behaviours in various captive environmental contexts (Benus et al. 1990, Koolhaas et al. 1999), but lately it has become clear that these individual differences also exists in all kinds of behaviours (Dingemanse and Dochtermann 2013). The most common use of the term 'animal personality' is the broad-sense definition, which defines animal personality as significant differences in the mean of any behaviour between individuals within the same population across environmental contexts (Reale et al. 2010a). One of the major questions is how these differences can persist within the same population? Traditionally, behavioural ecologists expect that selection for a single optimal level of behaviour in a specific environmental context would be favoured, and the possibility of persistent individual variation in animal personality is thus a puzzle that many researchers are working at solving.

One explanation might be that different behavioural types do better in different habitats or under different conditions (Sih et al. 2004b). For instance, highly active individuals may do better in predator-free habitats than less active individuals, because they will use more time on feeding rather than hiding. In habitats with predators, on the other hand, less active individuals may do better since they use more time hiding and thus avoid predators. For great tits (*Parus major*), fast explorers have been shown to be better during fierce competition, while slow explorers were better adapted when competition was reduced (Verbeek et al. 1994). Another possibility is that several behavioural types can be optimal in the same environment, as long as they all have the same lifetime fitness. . Thus, a bold individual that takes higher risks, might benefit by achieving greater access to food and mates, but might die at a younger age than a shyer individual that takes fewer risks (Reale et al. 2010b). The different behavioural strategies might also be under negative frequency dependent selection (Sih et al. 2004a), as in alternative reproductive strategies or producers and scroungers modelled by game theory (Bergmuller and Taborsky 2010). In addition, individuals may differ in their internal state due to stochastic events in their early development, which might affect their behaviour. Individuals that happen to start off in good condition may be able to afford to be more active and bolder than individuals in poorer condition, while individuals in poor condition have to 'make the best of a bad situation', and thus alternative state-dependent strategies develop and are maintained in order for all individuals of different states to best cope with their environment (Sih et al. 2004a).

Most behavioural traits are relatively plastic, and animals adjust their behaviours according to environmental and social context. For example, feeding behaviour might vary with the

level of competition, where a higher level of competition will lead to a more aggressive behaviour (Sih et al. 2004b). Any behaviour should therefore be studied across contexts to get a better understanding of plasticity and personality as attributes of the particular behavioural phenotype expressed by an individual (Dingemanse et al. 2010b). The statistical behavioural reaction norm (BRN) approach suggested by Dingemanse et al. (2010b) conceptually incorporates both behavioural plasticity and animal personality. BRNs show behavioural phenotypes over an environmental gradient for each individual, for instance aggression under different levels of social competition. The slope of the BRN therefore corresponds to the degree of behavioural plasticity for each individual, whereas the elevation corresponds to personality if it displays significant variation between individuals. Any consistent individual differences in behavioural plasticity (i.e. the degree of plasticity) could also be referred to as part of animal personality in terms of individual variation in responsiveness (Dingemanse et al. 2010b). Therefore, behavioural plasticity and animal personality can be regarded as two different aspects of a behavioural trait that can be selected on independently. They might also be linked (see Mathot et al. 2012), as has been observed in mice, where highly aggressive individuals showed lower plasticity over a social gradient than less aggressive individuals (Natarajan et al. 2009). The two parameters, slope and elevation, reflect the phenotypic behaviour of each individual, and it gives meaning to both inter-individual and intra-individual behaviour. Animal personality can therefore be studied alongside any plasticity in individual behaviour in different environmental contexts, both physical and social, and can be identified in the behaviour across a gradient of environmental contexts (Dingemanse et al. 2010b).

The interesting point here is that plasticity appears to have limitations, and each individual does not display the entire behavioural repertoire of the whole population (Reale et al. 2010a). This limitation in behavioural plasticity may essentially be what gives rise to animal personality, and this is difficult to explain in adaptive terms, since it would make sense for individuals to have the entire span of behavioural responses to any environment. One reason why all individuals in a population may not show the same optimal behaviour in every environmental context could be due to carry-overs between behaviours and/or contexts, meaning that the behaviour expressed in one context necessarily correlates with the behaviour in another (Sih et al. 2004b). For example, individuals that show a high level of aggression in competitive context might also show high aggression in another social context, like mating. This effect has been described elsewhere to explain seemingly maladaptive behaviours, such as sexual cannibalism and high activity in presence of predators. For both fishing spiders, *Dolomedes triton* (Arnqvist and Henriksson 1997), and funnel web spiders, *Agelenopsis aperta* (Riechert and Hedrick 1993), more aggressive females that show a (presumably beneficial) high attack rate on prey also had a higher attack rate towards conspecifics, and were more likely to (presumably maladaptively) attack approaching males that represent potential mates. For the fishing spider, this resulted in a lower percentage of fertile eggs and thus reduced fitness for such overly aggressive females (Arnqvist and

Henriksson 1997). Therefore, animal personality might only be explained by understanding the evolutionary and adaptive relationships between different behaviours and/or contexts.

Behavioural syndromes

As with correlations between the mean level of the behaviour and the degree of plasticity (Mathot et al. 2012), different behaviours have been found to correlate consistently over time and context for several species (Sih et al. 2004a). For example, bolder individuals tend to be more aggressive than shy individuals, and more exploratory individuals is often more active than less exploratory individuals (Riechert and Hedrick 1993, Verbeek et al. 1996). These suites of correlations between behavioural traits are called 'behavioural syndromes', and these correlations may also constrain plasticity and evolution to adaptive optima since one behavioural trait affects another (Mathot et al. 2012). As with limited plasticity, the adaptive value of these correlations can be hard to explain. Like the effect of carry-overs within behaviours between contexts, the correlations between behaviours within and between contexts might result in seemingly maladaptive responses. For example, it might be beneficial to have a high level of aggression in competition over both food and mates, but if this aggression then correlates positively with individual levels of activity it might prove costly in situations of high predation threat where less active, cautious individuals do best.

If two or more behavioural traits co-vary across time and contexts for all individuals in all populations, then these behaviours may well have evolved as a 'module', and should thus be studied as a functional unit rather than as series of single independent traits (Stadler et al. 2001). Differences in suites of behavioural correlations (i.e. the phenotypic variance-covariance matrix, or P-matrix, (Dingemanse et al. 2010a)) might occur not because there are constraints in the independent evolution of different behaviours, but because the specific pattern of correlations themselves might also be favoured by selection. This would mean that specific behavioural syndromes are not fixed for an entire species due to for instance genetic constraints, but can vary between different populations due to differences in environment and thus selection pressure. Suggestive evidence in this direction exists for three-spined sticklebacks (*Gasterosteus aculeatus*) in a study by Dingemanse et al. (2007, 2012). They showed that six stickleback populations from ponds with piscivore predators showed tight correlations between aggression, activity and exploration, whereas six stickleback populations from ponds without piscivore predators had only weak correlations or lacked it completely.

In behavioural syndromes, groups of behavioural correlations can be represented as vectors or axes along the line of correlation in multivariate behavioural space, providing latent variables that describe individual positions along this multi-dimensional axis (Dochtermann and Jenkins 2007, Dingemanse et al. 2010a). One well-established example of a behavioural syndrome is the proactive-reactive axis (Reale et al. 2010b). A proactive individual is more exploratory, aggressive and bolder than a reactive one, and they form routines and manipulate their environment to a higher degree. Thus, proactive individuals do better in

stable and predictable environments, while reactive individuals do better in changing environments (Coppens et al. 2010).

Innovation

As with animal personality, the study of innovation in animals was generally neglected by researchers in animal behaviour until about the last 20 years (Reader and Laland 2003), and like personality it had previously been regarded as an exclusively human characteristic. Innovation is defined as performing new or modified behaviours in new and different situations (Reader and Laland 2003), and it seems obvious now that animals also have the capacity to innovate. But innovation does not just involve one individual performing a new behaviour. It also requires that this new behaviour spreads throughout the population by the means of social learning, a process called cultural transmission (Reader and Laland 2000, 2003a). Most of the well-known examples of innovation in the animal world involve exploitation of a novel food or a new method for processing food (Reader and Laland 2003, Lefevre and Bolhuis, 2003). To be able to exploit new food sources is especially important for animals with opportunistic or generalist lifestyles, and a new food-processing method might reduce the energy costs per food unit. One of the first and best-known examples of innovation in animals is Imo the female Japanese macaque, *Macaca fuscata*, who was the first to start washing sand off sweet potatoes by submerging them in seawater before eating them, a behaviour that was later copied by the rest of the macaque population. Some years later, Imo also discovered that she could separate wheat from sand by putting the mixture in seawater and picking off the floating wheat grains, a behaviour that again spread throughout the population via cultural transmission (Kawai 1965). Other well known examples include milk bottle top opening by blue tits, *Cyanistes caeruleus*, that spread from the Midlands to much of the UK (Fisher and Hinde 1949), and tool-use in both birds (Rutz and St Clair 2012) and primates (Goodall 1964, McGrew 1992).

The time an animal uses to explore a new habitat or resource is time not used for feeding or mating in a familiar area. Hence, innovation carries costs in the form of time allocation at the very least. Innovation might also be costly in the means of increased predator risk, the risk of consuming hazardous food (Reader and Laland 2003) and reduced efficiency when utilizing familiar resources (Sol 2003). In addition, the large brains that are associated with higher levels of innovation (Wyles et al. 1983, Lefebvre et al. 1997, Reader 2003, Sol 2003) are known to be costly to produce and maintain, and require a longer time to develop (Aiello and Wheeler 1995, Kotrschal et al. 2013). Animals are therefore only expected to evolve large brains and innovate if the benefits outweigh these costs.

Innovation is widespread throughout many taxa, but it is not clear which individuals within a population are most prone to innovate. One suggestion is that different classes within a population differ in their innovation rate. For instance, females have been shown to be significantly more innovative than males for the guppies (*Poecilia reticulata*), which might be due to sex differences in the benefits of obtaining the resources needed for parental

investment, which is exclusively female in guppies. Female fecundity is significantly correlated with body size, thus female fitness depends heavily on food resources (Reznick and Yang 1993). Males, on the other hand, stop growing after they become mature, and their fitness is limited by the number of females they get to mate with, rather than their body size (Reznick and Yang 1993). This pattern of sex differences in innovation might also be true for other animals with skewed parental care and fitness effects of resource acquisition. State-dependent variables, like hunger level, also affect the innovation rate in animals. An individual that is starving might take the risk of eating a novel food if the alternative is dying of starvation. This has been shown for guppies where hungrier individuals were more innovative than individuals with a lower hunger level (Laland and Reader 1999a). Another study on guppies showed that the latency before younger males completed a novel task to get food was shorter than for older males (Laland and Reader 1999b). In guppies, body size correlates significantly with age, and during scramble competition younger, smaller males are less competitive, and hence they might be forced to explore more to find novel sources of food than older, larger males. The state of hunger, rather than the classification of age, could therefore explain the higher innovation rate (Laland and Reader 1999b).

Linking Personality and Innovation

Since certain individuals appear to differ in their innovation rate, and these differences are maintained across environmental contexts, it has been suggested that innovation may be linked to animal personality (Reader and Laland 2003). This would mean that the behavioural type of some individuals is linked with these individuals' innovation rate. Thus, there have been suggestions that innovation should be regarded as an aspect of personality, but there are few studies on this subject (but see Matzel et al. 2006 and Light et al. 2008), and most of those that have been carried out do not rule out confounding variables like the internal state of the individual, classification and environmental events (Reader and Laland 2003). These variables will all increase an individual's motivation and enhance the probability that those individuals attempt to and succeed at solving novel problems, and thus increase their innovation rate. As innovation also could be used as an alternative strategy for less competitive individuals in both scramble competition and mate competition, state, rather than differences in personality, may drive individual variation in innovation rate (Laland and Reader 1999a).

But it might be that innovation is linked to some specific behavioural types and not others. Bold or more risk-taking individuals might be expected to be more innovative than shy or less risk-taking individuals, because they approach and investigate novel objects and food at a higher rate. Hence, they will experience more novel situations in which new and flexible behaviour might be useful and carry some adaptive advantage. In addition, bold and more explorative individuals will tend to gather more information about their surroundings, which might be an important component of innovation. Conversely, bolder individuals have also been shown to be less flexible in their behaviour (Mathot et al. 2012). Dominant individuals

might be more aggressive than subordinates, and as such they can afford to be less innovative and thus avoid predation risk, and instead use their social access to limited resources to take advantage of the discoveries by low-ranking individuals in something akin to a producer-scrourer game (Katzir 1982).

Several studies on animal personality are methodologically quite similar to studies on innovation. Boldness is often measured as the time an animal uses before emerging from a refuge or latency before approaching a novel object (Laland and Reader 1999a). Approaching a novel object can also be measured as neophobia or neophilia, an important component of innovation (Webster and Lefebvre 2001, Reader and Laland 2003, Greenberg 2003). In the study by Dingemanse et al. (2007) on three-spined sticklebacks, the response to novel food was classified as an exploratory behaviour, but, as mentioned above, most of the innovative behaviour documented concerns the ability to utilize a novel food source. It could also work in reverse, where studies on innovation are equally good as animal personality studies. For example, in the study by Laland and Reader (1999a), innovation was measured as the latency before guppies went through a maze, where on the other side they were presented with a novel food. Individuals that were able to figure out the maze fastest and ate the novel food first were thus classified as more innovative than the slower individuals, but they could just as well have been classified as bolder or more exploratory individuals, or just more active. Thus, innovation appears to be confounded or to co-vary with several aspects of animal personality, and might therefore actually be considered an aspect of animal personality itself, or at least as a separate axis within a behavioural syndrome.

In this study, we will test if there is any behavioural syndrome within the guppy, and if these correspond to animal personality and innovation. We predict individuals with a higher activity level to explore more and be more innovative than less active individuals, as explained above. All individuals will be tested in isolation, and so there will be no scramble competition, and hunger levels will also be controlled for. Thus, we will examine if the guppies show individual personality by nature, rather than due to variation in a state-dependent variable like hunger level. Innovation will be measured as a product of each individual, and the cultural transmission aspect of innovation between individuals will therefore not be studied in this experiment. We will also see if different classes of individuals have more similar personalities, and if some of them are more prone to innovate. In the case of the guppies, this means different sex and size classes, as explained previously (Reader and Laland 2003). Here we predict that females will be more exploratory and innovative than the males, due to the skew in parental investment, and the fact that size has a higher effect on fecundity in female than in male. We also predict smaller individuals to be more exploratory and innovative than larger individuals, since their lower competitive ability should increase their motivation for searching for new food sources. Thus, developmental state rather than consistent individual differences might create differences in the behaviours. We will also see if there is any difference in innovation rate and personality between the three different populations with different selective histories in the wild.

Methods

Study species

The guppy is an ideal study organism in this case because there is a lot of information on their ecology in general as they have been used as study subjects in numerous research projects over many years (Magurran 2005). In addition, their innovation rate has already been studied (Laland and Reader 1999b, a, 2000), which makes it easier to replicate aspects of this study and link it to animal personalities. There have also been several experiments on the guppies here at NTNU over the last 10 years (Viken et al. 2006, Borg et al. 2012), and so there is a lot of background information on the three populations used in this study.

All the guppies used in this experiment are laboratory-reared from wild stock from the rivers Paria, Campo and Quare in Trinidad (10.7° N, 61.5° W), and the three populations have been kept separated in two large aquaria (400-500 l) per population. Approximately 500 individuals were collected from each river. The Paria population was collected in 1991 by G. Rosenqvist (Norwegian University of Science and Technology, Norway), and maintained at J.A. Endler's lab (Santa Barbara, USA). In 1994, 400 individuals from this population were transferred to the NTNU lab. The Campo and Quare populations were collected in 1998 by G. Rosenqvist, Å. Viken and I.A. Fleming (Memorial University, Canada). In late 1998/early 1999, there was an outbreak of fish tuberculosis in both the Campo and Quare populations, but only the Campo population got sick, which resulted in a bottleneck incident. Any selection due to the regular captive environment is expected to be the same for all three populations, and any between-population differences are therefore considered to have a historical genetic basis.

Experimental setup and video analysis

The light turned on at 09:00 am and off 09:00 pm each day. One male and one female guppy from each of the three populations were randomly captured from the population tanks and placed in three small isolation tanks (22x14.5x14 cm) - one for each population - seven days prior to the experiment. It was important to capture fish with different sizes and behavioural types at different times across the experiment to avoid any sampling biases within each population, i.e., the first fish captured at 'random' was not always the one used, since this might cause only the boldest fish or fish of a certain size to be captured earlier on in the experimental trials (Wilson 1998). The isolation tanks were placed in the same room as the experiment was conducted, and the water temperature in all tanks was 25°C. The experimental tanks (see Figure 1) were covered in black plastic to keep each individual in isolation. The fish were food deprived for seven days to increase motivation for activity in general and for eating the novel food, and to reduce any difference between individuals in hunger level. The final dataset contained 19 males and 19 females from each population, adding up to a total of 114 individuals.

All experiments were recorded by a Sony high-resolution colour CCD camera (model NC1381W), which was mounted above each experimental tank and adjusted to make the images as similar as possible. Every camera was connected to an H-264 Portable Mini Video Surveillance Recorder from LUPUS TEC (Recording resolution and frame rate: 704x576@25 FPS, 352x280@25 FPS (PAL)) with a 16 MbScanDisk memory card in it.

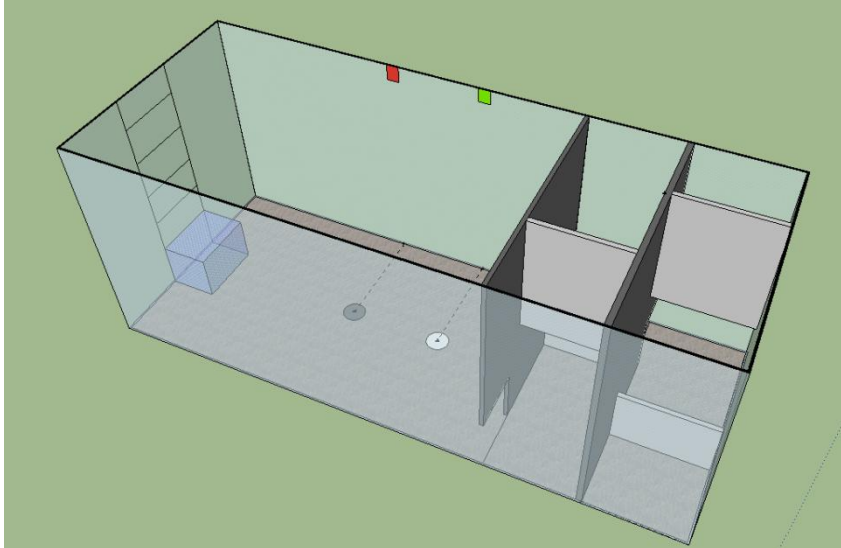


Figure 1. Tank set-up without the black plastic covering the walls. The tank was 61x25x24.5 cm, and water was filled up to 18 cm. The maze consisted of two partitions that ran across the width of the tank 10 cm apart, with a hole (6x6 cm) at the bottom at opposite corners. Between these two partitions and between the inner partition and the glass wall, four additional partitions were fitted. The first and the third additional partitions were mounted 4 cm above the ground, while the second and fourth were 5 cm and 8 cm from the ground up, respectively, thus making the maze three-dimensional. The white spot indicates the position of the novel object, while the grey spot indicates the position for the novel food. In the tank-set up, these locations were indicated by the pieces of green and red tape on the top of the glass wall. The blue box refers to the acclimatization cage – see the text for more details. The maze door, not included in this figure, consisted of a plate placed right in front of the first partition of the maze.

Exploration

At 09:15 each day the three male and three female guppies were randomly assigned to one of the six experimental tanks (see Figure 1) in acclimatization cages. These cages provided a small area of dark shelter where the water could flow through, in which the guppies got ten minutes to settle. The cameras were then turned on and the fish was gently encouraged to leave the cage, and the door was closed behind them to prevent re-entry. The fish were then given 30 minutes to move about and possibly explore the open area. In the video analysis, a

grid of 24 squares was drawn on a transparent sheet and placed on the TV-screen as shown in Figure 2. Exploration was quantified by counting the number of new squares entered per 5 minutes at the start, middle and end of the 30 minutes period, as well as the time to reach this total. Since most of the fish explored all 24 squares, the variable with maximum squares explored was highly skewed, and thus did not contain much variation. The time to reach the maximum number of new squares was therefore divided by the total number of new squares explored to make a rate representing the time taken to explore each square, a low rate indicating fast exploration. In addition, activity was measured as the total number of squares visited in the same 5 minutes intervals. The video analysis started at the point where the surface water was relatively still, and thus not disturbed by any flickering lights. This was done for all four tests.

Maze

After 30 minutes of exploration, the maze door was removed by lifting it straight up, and the guppies were encourage to enter the maze using a small landing net drawn slowly through the water (note that this door is not illustrated in Figure 1). The maze door was then replaced, and the guppies had 30 minutes to get to the end of the 3-dimensional maze. There was no reward, like food or a hiding place, at the end of the maze, hence the maze only represented innovation in terms of moving in a novel way through a novel environmental set-up (see Laland and Reader, 1999a). Thus, both the exploration trial (above) and the maze constitute spatial tasks. Innovation was assessed by how far into the maze each fish moved and the time it took to reach the maximum point. A grid of 24 squares was placed along the maze and laid over the screen during video analysis to measure the maximum distance reached (i.e. 2 squares per steps and 12 steps) and the time taken to reach the maximum distance (see Figure 2). Again, the variable for the maximum point reached was highly skewed, because most of the guppies completed the maze, and so a ratio with time to reach maximum point divided by maximum point reach was created to represent a rate of movement through the maze. Activity was measured as movement between squares in the same 5 minutes intervals as in the exploration test, although note that the smaller square sizes in the maze required further correction to allow comparison with activity in the main tank area.

Novel object

After another 30 minutes, the guppies were removed from the maze with a landing net and moved back into the main exploration area. Here they were presented with a novel object, an aquarium decoration, which was placed in the middle of the exploration area; 10 cm from the maze wall (see Figure 1). The investigation of the novel object was recorded as the minimum distance and the time taken to reach this distance. This was done by drawing 5 concentric circles 1 cm apart on a transparent sheet placed on the TV-screen around the novel object during video analysis of this part of the trial, as shown in Figure 2. As with the previous tests, most guppies completed the task, and therefore a ratio with time to reach minimum distance divided by minimum distance reach was created to represent the rate of

approach towards the novel object. If the guppies did not enter any of the circles, they were given a score of one, while if they crossed the first circle they were scored with a value of two, and so on to the innermost circle where they were scored a value of 6. Activity was measured using the same grid as in the exploration trials (above), but in this case for the first, middle and last 3 minutes, and the data were then scaled up to equivalent values for 5 minutes for comparison in the other activity measures.

Novel food

15 minutes after the novel object had been placed in the tank, it was removed and the guppies were presented with salmon food in a glass bowl, which was a novel food for these populations. The glass bowl was placed into the gravel before the trial started, thus should not represent a novel object in itself. It was placed in the middle of the tank, 10 cm from where the novel object had been, as indicated in Figure 1. They had 30 minutes to complete the task, and the time before the guppies approached and possibly ate the novel food was recorded using the same method as for the novel object, with 1 cm concentric circles on the monitor screen during video analysis. Again, a ratio with time to reach minimum distance divided by minimum distance reached was created to represent a rate of approach towards the novel food. Activity in this test was measured in the same way as in the exploration and novel object tests (above).

The exploration of the new area and of the novel object can here be seen as traditional personality aspects of exploration and boldness and shyness (Sih et al. 2004a), while the maze and the novel food represent innovative behaviours (Laland and Reader 1999a). Alternatively, the exploration test and the maze can be seen as spatial tests, while approach of the novel object and novel food can be seen as non-spatial tests.

Table 1. Description of the different variables from each treatment that was used in the analysis. All time units are in seconds.

Treatment	Observed variable	Description
Exploration	Expl act	Total number of squares visited, were the three measurements (first, middle and last) were added together.
	Expl rate	Time to explore maximum number of new squares divided on number of new squares explored.
Maze	Maze act	Total number of squares visited, were the three measurements (first, middle and last) were added together.
	Maze rate	Time to reach maximum level in the maze divided by maximum level reached.
Novel object	NO act	Total number of squares visited, were the three measurements (first, middle and last) were added together.
	NO rate	Time to reach minimum distance to the novel object divided by minimum distance reached.
Novel food	NF act	Total number of squares visited, were the three measurements (first, middle and last) were added together.
	NF rate	Time to reach minimum distance to the novel food divided by minimum distance reached.

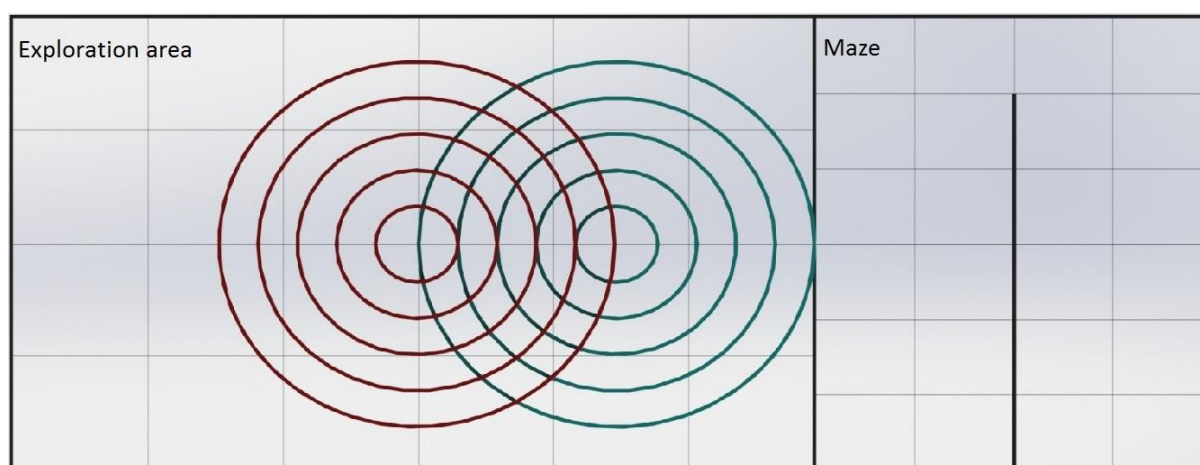


Figure 2. The tank seen from above with the grids for the video analysis. Both the exploration area and the maze were divided into 24 squares to measure activity and exploration. The maze is also divided into 12 steps (2 squares per step) to measure the maximum distance reached by each guppy. Concentric circles were placed over both the novel object and the novel food to measure approach. The blue rings show the novel object and the red rings show the novel food.

Statistics

All the statistical analyses were carried out in R, version 3.1.1 (R Core Team 2015). Raw data was used in all the analyses except for repeatability and habituation. Here all three activity measurements from each of the four tests were used, and they had to be square-root transformed to satisfy the assumption of normality.

After analysing all the videos, both observers re-watched eight trials from the start, middle and end to control for any within-observer error caused by any changes in measures due to experience. Both observers also watched 16 of the same videos to control for any between-observer error. The correlation between these different measures ranged from $r = 0.97$ to 1.00 within observer and $r = 0.98$ to 1.00 between observers. Thus, all observations were highly repeatable. Observer identity was also added to all the models to check if it had any effect, but removed from all results presented as it was shown to be highly non-significant. The same procedure was carried out for experimenter identity, i.e. who conducted the experiment on a particular day. Experimenter identity had a significant effect on some variables, but this was caused by the fact that both experimenters conducted the experiment on the first day, which only included four guppies. When these few fish were removed, experimenter was non-significant, and since day of experiment had no significant effect, experimenter was removed from all analyses presented. Experimental tank identity (A-F) was also added to all the models, and was significant in only one case (once multiple tests were taken into account) – for the activity measurements in the exploration trials, individuals were significantly less active in tank D compared to the others ($F_{5,108} = 4.04$, $p = 0.001$). The lack of any systematic effect here suggests that this tank identity effect probably had minimal influence on the main results, especially since tank identity was randomized between sexes and populations. In addition, tank had no effect on the syndrome structure, and was therefore removed from all the model results presented.

As mentioned, female guppies are larger than males (Magurran et al. 1995), and this had to be taken into account when testing for effects of sex along with an effect of size. This difference was highly significant in this dataset ($F_{1,108} = 7.68$, $p < 0.001$), with an average length of 20.14 ± 4.03 mm for the females and 15.73 ± 1.56 mm for the males. There was also a significant difference in variance in size between the two sexes ($F_{1,56} = 6.68$, $p < 0.001$), with the variance within females being larger than within males. There were no significant differences in size between the populations in total ($F_{2,108} = 2.53$, $p = 0.08$), or between males or females from the different populations, i.e. no effect of the interaction between sex and population ($F_{2,108} = 1.89$, $p = 0.16$).

Repeatability and habituation

Activity was measured at three times within each treatment (first, middle and last 5 minutes), and was used to see if there was any habituation (a reduction in activity over time) and repeatability within each fish for this behaviour, hence if the guppies showed personality in activity level. Repeatability is defined as the phenotypic variance explained by individual identity (Dingemanse et al. 2002), and is calculated by dividing the between individual variance by the total variance (Lessells and Boag 1987).

$$r = \frac{\sigma_{among}^2}{\sigma_{among}^2 + \sigma_{within}^2}$$

Thus, it varies from zero to one, and the less within individual variance the higher the repeatability. In this analysis, a mixed-effect model in the rpt2 package was used to estimate repeatability with confidence intervals. The twelve different periods of activity measurement (three measurements from each of the four treatments) was added as a fixed effect to control for any difference in activity level due to the different tests and habituation over time. Parametric boot-strapping based on model estimates was then used to derive a distribution of repeatability estimates. In the habituation model, the twelve periods were added as continuous 'time' variable to see how the activity level changed over time, while the four different tests were added as a factor. Sex and body size was also added to the model to see if they affected the rate of habituation. The full model contained a lot of non-significant interactions, so a model simplification procedure based on p-values and AIC-values was performed (see Forstmeier & Schielzeth 2011 - see Appendix A). Only the final model with all the two-way interactions is presented here.

In both the repeatability model and the habituation models, fish identity was added as a random effect, and the activity variables were square-root transformed to avoid heteroscedasticity. In addition, they were both run with and without the maze, since the maze represents a somewhat different environment, confounding both the repeatability and the habituation.

ANCOVA

Univariate ANCOVAs were carried out separately on all the eight behavioural variables (see Table 1) to see if there were any differences between the three populations, sex or size, or interaction effects between any of these. Because of the difficulties with appropriate methods of model simplification and the associated problem of an increased probability of false-positives (Forstmeier and Schielzeth 2011), the full models with all interactions are presented in all cases.

Correlations between variables

Bivariate correlations between the eight different behavioural variables were calculated using Pearson's correlation coefficients. This correlation matrix was produced for the overall results, and for the two sexes.

Structural equation modelling

To further study the structure of the co-variances between the different behaviour variables, structural equation modelling (SEM) was used to examine seven hypothesized covariance structures with different underlying latent variables (see Table 2). This was carried out in the lavaan package in R. The co-variation between the latent variables was retained in all models except H0.

Table 2. Description of the seven different models tested in SEM – see predictions above in Introduction

Hypothesis	Description
H0 – no correlations	No correlations between any of the variables
H1 – all correlates	All variables loads onto one latent variable
H2 – personality vs. innovation	Animal personality and innovation constitute two different latent variables
H3 – spatial vs. non-spatial	Spatial and non-spatial tasks constitute two different latent variables
H4 – activity driven	The entire syndrome is driven by activity where all the activity variables constitute a latent variable, and in addition correlate with the rate variable within the same test.
H5 – activity vs. rate	Activity and rates constitute two different latent variables
H6 – only activity	Only the four activity variables constitute one latent variable

Models were ranked according to the Akaike information criterion (AIC), and the best model was used further to see if there were any structural differences between sexes and among populations, and to see if there was any effect of body size. The model was run separately for each sex and population, with both free parameters and with parameters constrained to the factor loadings of the opposite sex or the two other populations (N. Dochtermann pers.com). To see if there was any effect of body size on the syndrome structure, the best model was run with only the variance term for body size and with body size as part of the behaviour syndrome(s) (i.e. the latent variables). The AIC values were again used to see if the models were statistically different from each other.

Results

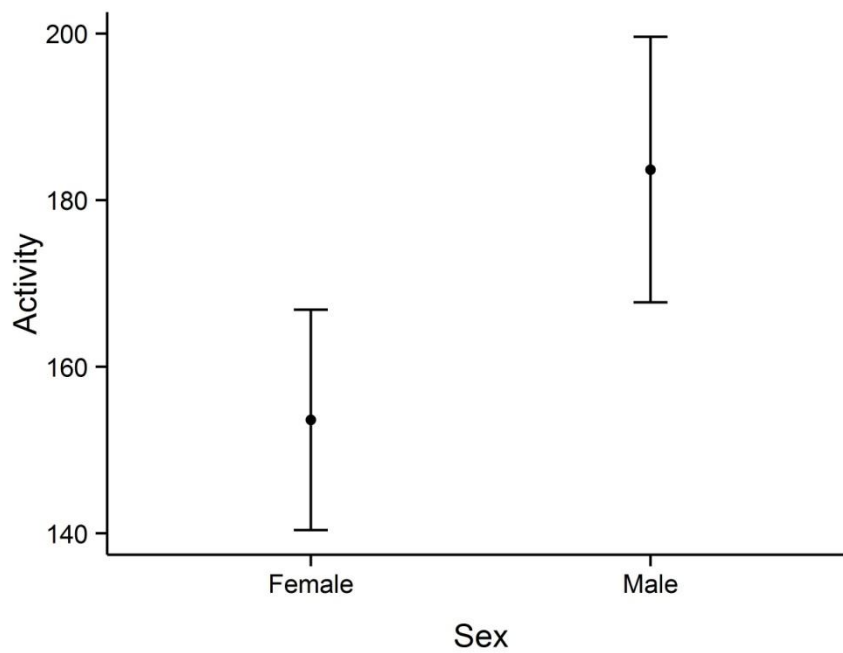
In the separate univariate tests of the variation in each of the eight response variables, there were very few significant effects, especially taking into account the number of tests (i.e. $p < 0.01$ in Table 3). The exception here was that sex and body size had significant effects on activity in the novel food test, where males had a higher activity level than females and smaller individuals were somewhat more active than larger ones, but an interaction term showed that this size effect was only the case within females (Figure 3).

Table 3. The effect of sex, population, body size and their interactions on the eight behaviour variables. The highly significant effects ($p < 0.01$) are shown in bold, while the various moderately significant effects ($p < 0.05$) are in frames. The effect degrees of freedom are given in each case, and the error degrees of freedom are 102 in all models.

Activity variables									
	df	expl_act		maze_act		no_act		nf_act	
		F	P	F	P	F	P	F	P
Pop	2	1.14	0.324	1.32	0.272	0.85	0.429	1.77	0.175
Sex	1	4.90	0.029	3.74	0.056	0.73	0.395	7.42	0.008
Size	1	5.02	0.027	3.53	0.063	3.55	0.062	8.05	0.005
Pop:sex	2	1.28	0.284	0.67	0.512	0.16	0.856	1.51	0.226
Pop:size	2	0.48	0.618	0.54	0.585	0.26	0.775	1.14	0.323
Sex:size	1	4.72	0.032	3.76	0.055	0.63	0.430	7.19	0.009
Pop:sex:size	2	1.32	0.272	0.64	0.531	0.13	0.874	1.27	0.284

Rate variables									
	df	expl_rate		maze_rate		no_rate		nf_rate	
		F	P	F	P	F	P	F	P
Pop	2	1.85	0.163	0.18	0.836	0.79	0.455	1.62	0.203
Sex	1	0.99	0.321	6.74	0.012	0.86	0.357	0.08	0.773
Size	1	0.16	0.693	0.01	0.937	0.88	0.351	0.43	0.512
Pop:sex	2	0.02	0.979	3.92	0.023	2.28	0.108	0.83	0.438
Pop:size	2	1.71	0.187	0.24	0.788	0.47	0.626	1.56	0.215
Sex:size	1	1.21	0.273	5.98	0.016	0.63	0.428	0.15	0.695
Pop:sex:size	2	0.06	0.939	3.71	0.028	2.24	0.111	0.70	0.501

a)



b)

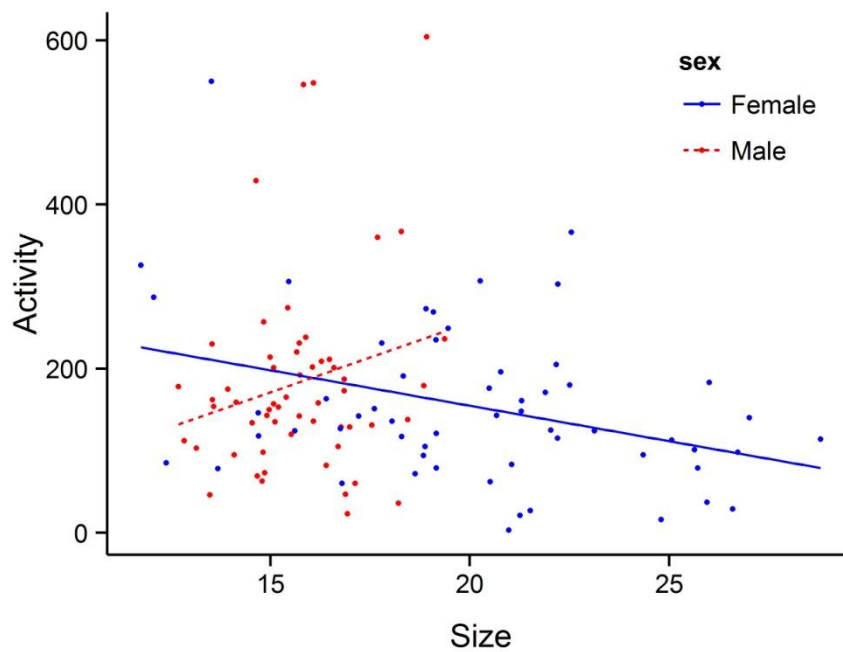
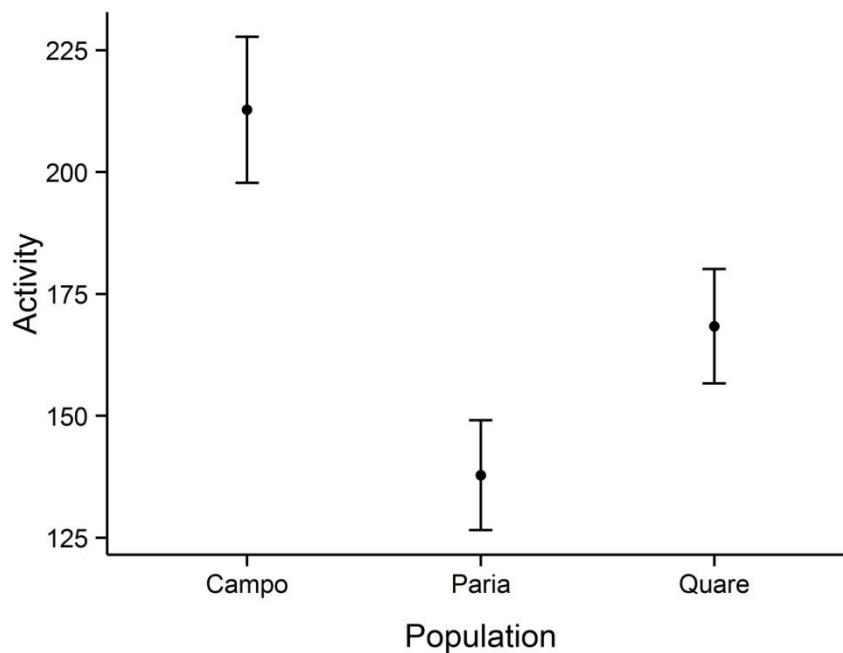


Figure 3. Activity level (quantified as number of squares visited) during the novel food test, shown as a) average activity level (mean±SE) for males and females; and b) regressed against body size for females (blue dots and line, $y = -8.62x + 327.27$) and males (red dots and line, $y = 16.94x - 82.81$). When activity during the novel food test was tested independently for the two sexes, there was no effect of size in males ($F_{1,55} = 7.59$, $p = 0.10$), but a highly significant effect in females, with smaller females being more active ($F_{1,55} = 7.59$, $p = 0.008$).

From the univariate tests, it was clear that that population had no significant effect on any of the measured behaviours when in the full model (see Table 3). However, when population was the only explanatory variable included, it dropped out as highly significant for all the activity measurements ($F_{2,111} = 8.70$, $p < 0.001$) and for the rate of exploration ($F_{2,111} = 5.50$, $p = 0.005$), shown in Figure 4. The guppies from the Campo population showed a higher activity level for all the activity measurements, while guppies from Paria had the lowest activity level. Guppies from Paria also showed the fastest rate of exploration, while guppies from Campo had the slowest. Therefore, although these results should be viewed with caution, because they are clearly confounded by effects of sex and population size (Table 3), they do suggest some meaningful differences between the populations in activity and exploration in the first treatment.

a)



b)

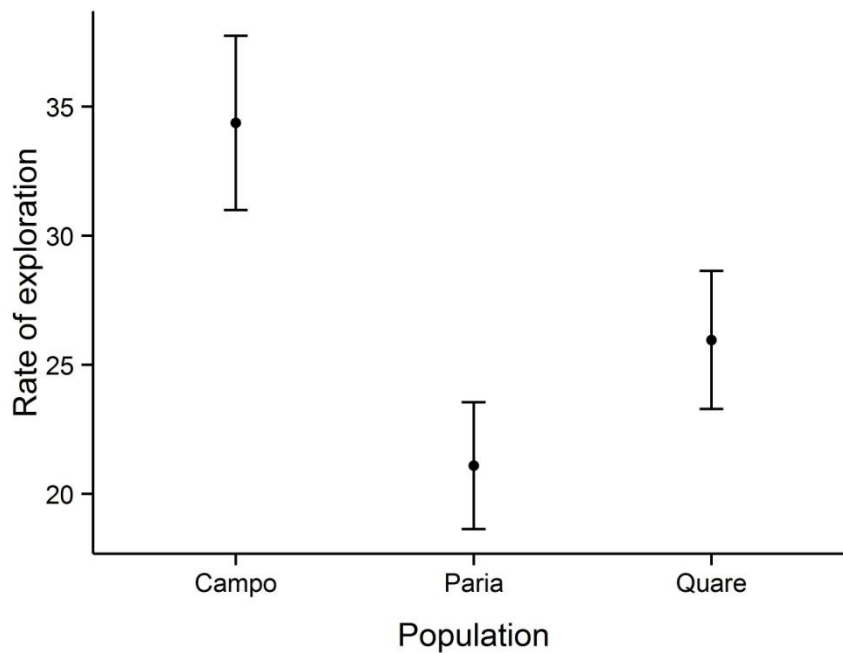


Figure 4. The difference between the three populations for a) the average activity level (mean \pm SE), quantified as number of squares visited, combined for all four treatments; and b) the rate of exploration between the three populations. Guppies from Paria have the lowest activity level, but the fastest exploration rate, while guppies from Campo have the highest activity level and slowest exploration rate.

Activity levels were highly individually repeatable for the different populations and sexes (see Table 4). This suggests that individual guppies differed in their activity levels, and that these differences were individually consistent over the limited time of these trials. Repeatability was calculated both with and without the maze, since the maze represents a different environment. As this had very low effects on the results, it seems that guppy activity was highly individually repeatable both within and between these slightly different environments.

Table 4. Repeatability (R) in individual activity calculated for combinations of the different populations and sexes. Since the maze is a somewhat different environment, it does not strictly represent a repeatable measure *per se*, and so the results are shown with and without the maze data included. The calculations were carried out using the function rpt2 in the rpt package in R, and fitted with a linear mixed effect model with the 12 replicates (3 time intervals for each of the 4 treatments) as a fixed effect and fish identity as a random effect. Activity was square root transformed. The confidence intervals (CI) are provided in brackets

Population	Sex	R (CI) with maze	R (CI) without maze
Paria	Both	0.516 (0.433-0.577)	0.526 (0.438-0.601)
Paria	Male	0.545 (0.430-0.622)	0.543 (0.408-0.641)
Paria	Female	0.480 (0.358-0.575)	0.498 (0.361-0.606)
Campo	Both	0.450 (0.362-0.527)	0.516 (0.423-0.597)
Campo	Male	0.503 (0.388-0.604)	0.555 (0.434-0.656)
Campo	Female	0.386 (0.265-0.496)	0.467 (0.339-0.581)
Quare	Both	0.427 (0.340-0.499)	0.443 (0.346-0.530)
Quare	Male	0.424 (0.292-0.533)	0.460 (0.309-0.576)
Quare	Female	0.429 (0.311-0.538)	0.390 (0.242-0.528)
All	Male	0.518 (0.452-0.570)	0.543 (0.472-0.603)
All	Female	0.475 (0.409-0.526)	0.498 (0.418-0.565)
All	Both	0.501 (0.456-0.537)	0.527 (0.477-0.568)

From the habituation model, there was no effect of the interaction between time and the four different tests (see Table 5), thus the slope is not significantly different in the four tests (see Figure 5). This means that the graph never flattens out, and the guppies never fully habituated to the new environment during the course of these tests. The effect of time is significant, however, and it is clear that the activity level keeps dropping over time (-1.78 ± 0.041), indicating that these fish were in the process of habituating. The difference between the mean levels of activity between the four tests was highly significant, though mostly due to the maze test. When maze was excluded this effect was highly reduced, but still present, again indicating that activity levels were still progressively dropping down to some presumed base-line level once habituated. When the guppies were moved back into the exploration area after they had been in the maze, their activity level increased to a level close to and even above that compared to before they entered the maze. This could indicate new exploration to see if there are any changes in this formally familiar area. Activity level then quickly drops back down again, but this shows that the maze test disturbed the habituation process in these guppies.

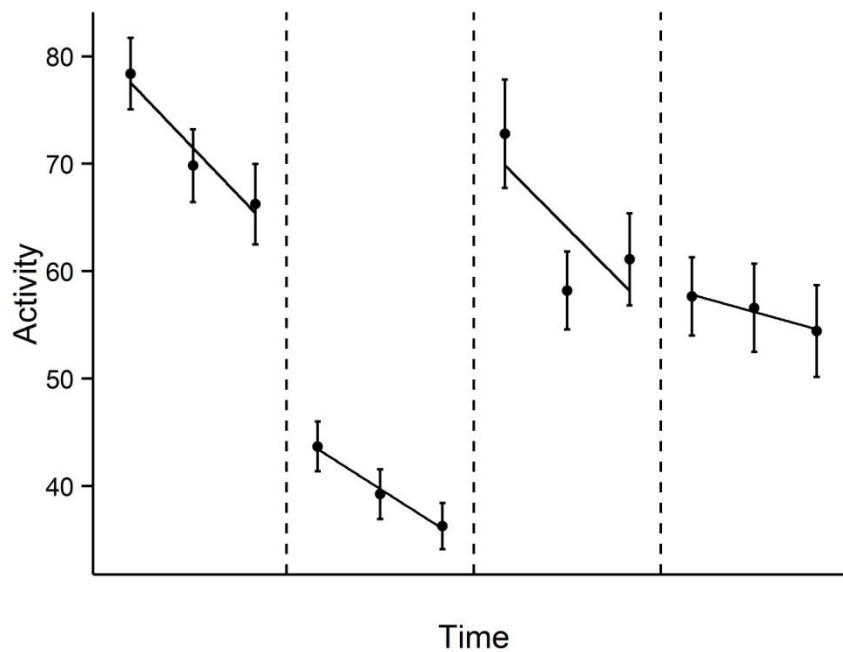


Figure 5. Change in activity level within and between the four different tests over time. From left to right: exploration, maze, novel object and novel food. The slope is not significantly different between the tests, which mean that the overall activity level does not drop to a base level, and the guppies do not habituate. However, the negative slope indicates that they are in the process of habituating.

The highly significant interactions between body size and time, and between body size and test, clearly show that size had an effect on the rate of habituation (see Table 5). Here, the larger individuals habituated at a faster rate than smaller individuals, which might be the reason why smaller fish are shown to have a higher activity level (Table 3, Figure 3b). However, the effect size here was not particularly strong (0.073 ± 0.019).

Table 5. Results from the habituation model including test, time, body size, sex and all the two-way interactions as fixed effects, and fish identity as a random effect (see Appendix A for model reduction procedure). Activity was square root transformed. The degrees of freedom were calculated with a Satterthwaite approximation, and both the effect degrees of freedom and error degrees of freedom are given in each case. Significant effects ($p < 0.01$) are in bold, while almost significant effects ($p < 0.05$) are in frames. The significant interaction between body size and time shows that size affects the rate at which the individuals habituate, where larger individuals habituate faster than smaller individuals. The sample size was 1368 for the model including the maze and 1026 for the model without the maze. The reduction in sample size in the model without the maze might be the reason for reduced significance for several of the variables.

Variables	With maze			Without maze		
	df	F	p	df	F	p
Test	3, 1239	7.44	<0.001	2, 901	4.95	0.007
Time	1, 1239	20.26	<0.001	1, 901	14.04	<0.001
Size	1, 823	7.32	0.007	1, 867	5.91	0.02
Sex	1, 138	7.67	0.006	1, 149	6.76	0.01
Test:Time	3, 1239	0.85	0.47	2, 901	1.16	0.31
Test:Size	3, 1239	6.82	<0.001	2, 901	5.68	0.004
Test:Sex	3, 1239	0.79	0.50	2, 901	0.55	0.58
Time:Size	1, 1239	14.23	<0.001	1, 901	9.91	0.002
Time:Sex	1, 1239	1.76	0.18	1, 901	0.95	0.33
Size:Sex	1, 110	6.47	0.01	1, 110	6.22	0.01

The individual repeatability in activity can also be seen in the high positive correlations between the four different activity variables (see Table 6). Most of the pair-wise correlations between the activity variables and rate variables are negative, showing faster exploration or approaches by more active individuals. The correlations between the rate variables are mostly positive, but most of these are not strong or significant.

Table 6. Pair-wise correlations between the eight behavioural variables. The significant correlations are marked in bold, while correlations within the same tests are in frames.

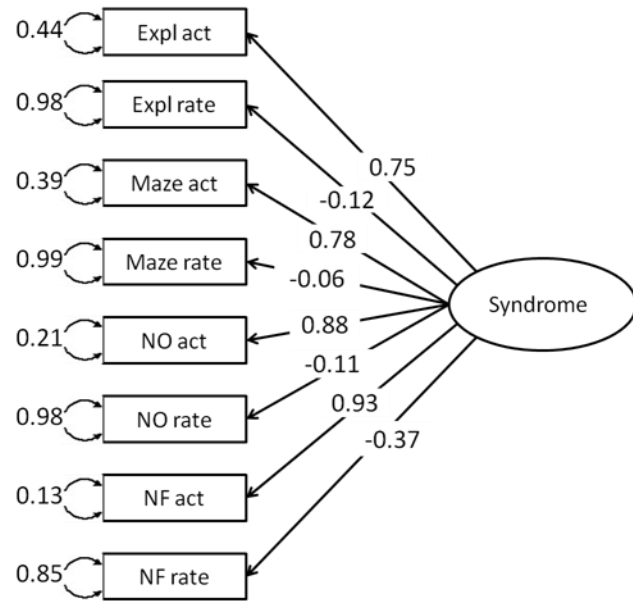
	Exploration		Maze		Novel object		Novel food	
	Activity	Rate	Activity	Rate	Activity	Rate	Activity	Rate
Exploration activity	1	r=-0.10 p=0.31	r=0.63 p<0.001	r=-0.09 p=0.33	r=0.66 p<0.001	r=-0.11 p=0.24	r=0.68 p<0.001	r=-0.34 p<0.001
Exploration rate		1	r=0.02 p=0.87	r=0.19 p=0.04	r=-0.11 p=0.25	r=0.12 p=0.21	r=-0.12 p=0.19	r=0.19 p=0.05
Maze activity			1	r=-0.13 p=0.16	r=0.68 p<0.001	r=-0.03 p=0.76	r=0.73 p<0.001	r=-0.22 p=0.02
Maze rate				1	r=0.03 p=0.77	r=0.27 p<0.001	r=-0.05 p=0.57	r=0.07 p=0.43
Novel object activity					1	r=-0.12 p=0.21	r=0.83 p<0.001	r=-0.35 p<0.001
Novel object rate						1	r=-0.08 p=0.38	r=0.11 p=0.23
Novel food activity							1	r=-0.34 p<0.001
Novel food rate								1

The correlation matrix in Table 6 is difficult in itself to interpret as a whole, and so it was applied in the different SEM models to ascertain the particular syndrome structure. The H0 hypothesis, where none of the observed variables correlates, has the worst fit (see Table 7), which indicates that there is some sort of syndrome involving these variables. Of the four first models, presented in Figure 6, there is no statistical difference between H1 (all correlates), H2 (personality vs. innovation) and H3 (spatial vs. non-spatial), thus, it is hard to say which syndrome explains the (co-)variance best. Also, these syndromes might only be driven by the substantial co-variance between the latent variables due to the high correlation between the activity variables.

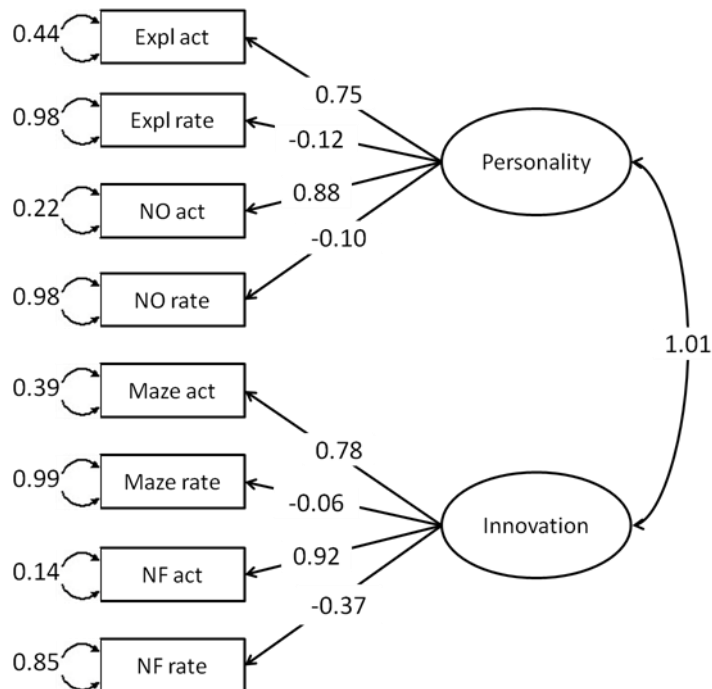
H0 – no correlations



H1 – all correlates



H2 – personality vs. innovation



H3 – spatial vs. non-spatial

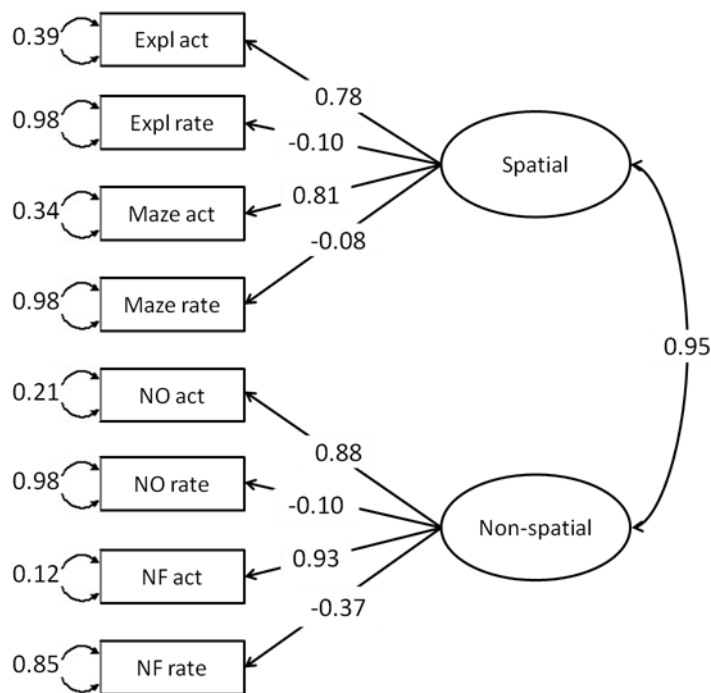
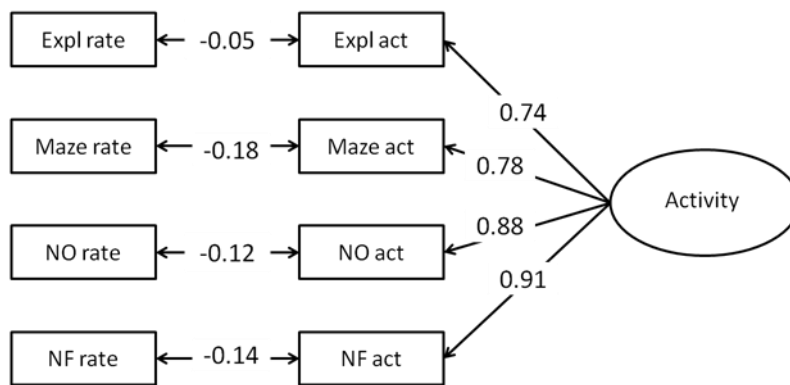


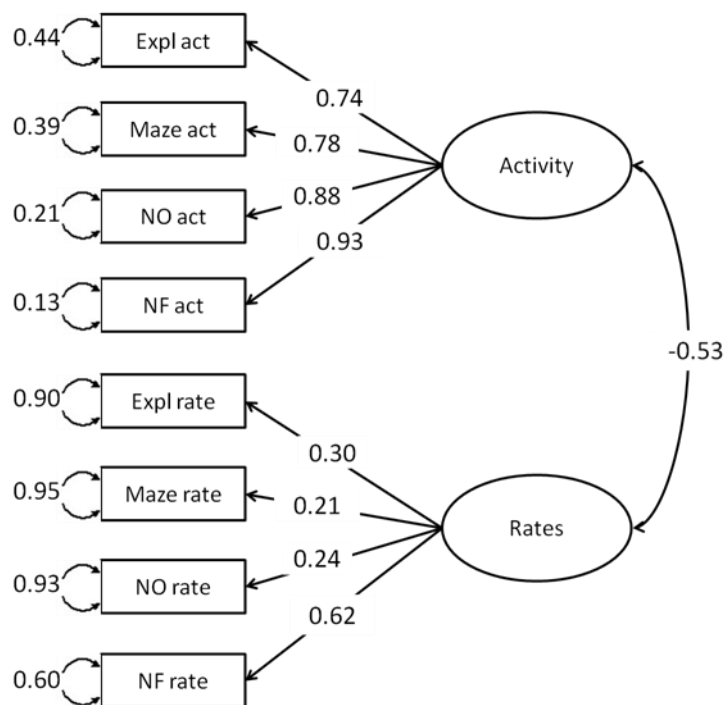
Figure 6. Causal diagrams of the four original hypotheses from Table 2. Standardized factor loadings are shown on the one-headed arrows. The double-headed arrows shown next to each of the observed variables (squares) show the error variance left unexplained by the latent variable (circles), while the double-headed arrow between the latent variables show the correlation between them. The different models shown are: H0 – no co-variance between any of the observed variables – i.e. the null model; H1 – all observed variables load onto one latent variable, so everything co-varies with everything; H2 – the co-variation separates into two latent variables, animal personality and innovation; H3 – two latent variables, spatial and non-spatial, separate the behaviour variables.

It seems unclear whether any of the SEM models H0-H3 have really captured the actual patterns of co-variance in the data. However, we know that there was a high repeatability and correlations between all the activity variables, and that things are made less clear by the weaker correlations between the rates variables, and also between the rates and activity variables (Table 6). Therefore, exploratory principal component analyses (PCA's) were conducted to further investigate possible syndrome structures not yet considered (see Appendix B). The PCA's were run without a rotation and with a varimax rotation, and from what we discovered from these two PCA's, three new models were constructed and tested in SEM, as presented in Figure 7.

H4 – activity driven



H5 – activity vs. rates



H6 – only activity

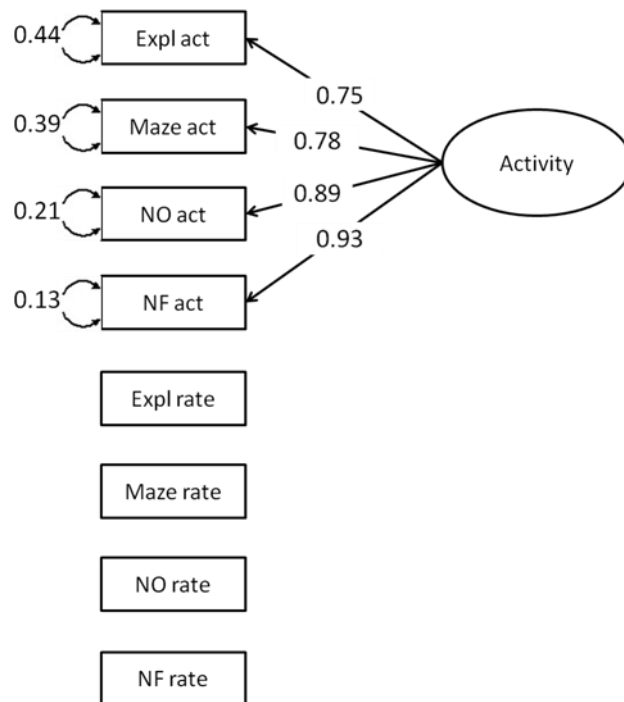


Figure 7. Causal diagrams for the three additional models described in Table 2, inspired from the results of PCA (Appendix B). Standardized factor loadings are shown on the one-headed arrows. The double-headed arrows shown next to each of the observed variables (squares) show the error variance that is left unexplained by the latent variable (circles), while the double-headed arrow between the latent variables show the correlation between them. The models shown are: H4 – activity constitute a latent variable while it correlates with the rate variable within the same test, thus the syndrome is driven by activity; H5– activity and rate as two separate latent variables, which is based on the un-rotated PCA; H6 – activity as the only latent variable, which is based on the varimax rotated PCA.

H5 (activity vs. rate) emerges as the best model (Table 7), meaning that the rate variables were not simply driven by levels of activity during the different treatments. The factor loading for activity are higher than those for the rates, indicating that the activity has a stronger effect on the syndrome, although there is obviously some separate effect of the rate variables as well, which may or may not co-vary within themselves. The co-variance between the latent variables is negative, as was expected, since more active individuals are more likely to complete the tasks, e.g. explore a new area or get through the maze, faster.

Table 7. Comparison of the seven SEM models (see Table 2 and Figures 6 and 7) using AIC values, where K is the number of parameters estimated. The H5 (activity vs. rates) is significantly better than the other models.

Model	Name	K	AIC	ΔAIC
H5	Activity and rates	17	2283.92	0.00
H3	Spatial and non-spatial	17	2287.59	3.67
H1	All correlates	16	2287.68	3.76
H2	Personality and innovation	17	2289.59	5.67
H6	Only activity	12	2298.15	14.23
H4	Activity driven	16	2300.85	16.93
H0	No correlations	8	2596.11	312.19

For both the Campo and Quare populations, the models with free parameters had the highest AIC value (see Table 8) meaning that the factor loadings from the other populations could explain the (co-)variance just as well as freely estimated parameters. For the Paria population, however, the model with free parameters had the best fit, but was hardly distinguishable from when it was constrained with the parameters for Quare. Thus, it appears that there were no significant differences in syndrome structure between any of the three populations.

Table 8. A comparison for the three populations between model H5 (activity vs. rate) with free parameters and parameters constrained to the factor loading of the two other population. K is the number of parameters estimated.

	Paria		Campo		Quare	
	K	AIC	K	AIC	K	AIC
Free parameters	17	742.54	17	788.47	17	783.18
Constrained parameters (Paria)	-	-	11	789.20	11	777.64
Constrained parameters (Campo)	11	757.13	-	-	11	779.83
Constrained parameters (Quare)	11	742.81	11	785.49	-	-

When H5 (activity vs. rates) was run separately for the two sexes, the model would not converge properly for the male subset, thus it was not possible to compare the syndrome structure between the two sexes for the best model. Given that the sample sizes for the two sexes was the same, it might well be that it is a difference in the co-variance matrices that enables us to run H5 for females but not for males. When looking at the correlation matrices for the two sexes we can see that the correlations for some of the rates are somewhat lower

in males than in females (see Appendix C), and these low correlations suggest a different structure, and might also cause the model to collapse when run on the subset for males. The comparison of males and females was possible for the H6 model (only activity), where the models with free parameters had higher AIC values (1139.59 for males and 1176.76 for females) than the models with constrained parameters (1136.93 for males and 1173.29 for females) for both sexes, thus no difference in syndrome structure in activity between males and females.

To see if body size had any effect on the syndrome structure, this variable was added as only a variance term, as part of either the activity syndrome or the rate syndrome, and alternatively as part of both syndromes. As can be seen from Table 9, including body size as part of the activity syndromes gives the best fit. It is, however, not statistically significant from the models where body size is part of the rate syndrome or both syndrome, but it is significantly better than with only the variance term, indicating that body size did affect the syndrome structure in the guppies in some way. Size had a negative factor loading (see Figure 8), which confirms the effect seen above (Table 3, Figure 3b) that smaller individuals were more active than larger individuals, suggesting that the behavioural syndrome identified here may be condition-dependent in terms of level of individual growth and/or development.

Table 9. Body size was included in the best model (H5 – activity vs. rate) as just a variance term or as part of either the activity syndrome or the rate syndrome or both to see if it had any effect on the syndrome structure. The model with the best fit is the one where body size is included as part of the activity syndrome, and this model is significantly better than the model where only the variance term for body size is included. It is, however, not significantly better than when size is added as part of the rate syndrome or both syndromes. K is the number of parameters estimated.

Model	K	AIC	ΔAIC
Activity syndrome	19	2606.22	0.00
Rate syndrome	19	2607.00	0.78
Both syndromes	20	2608.00	1.78
No syndrome	18	2608.43	2.21

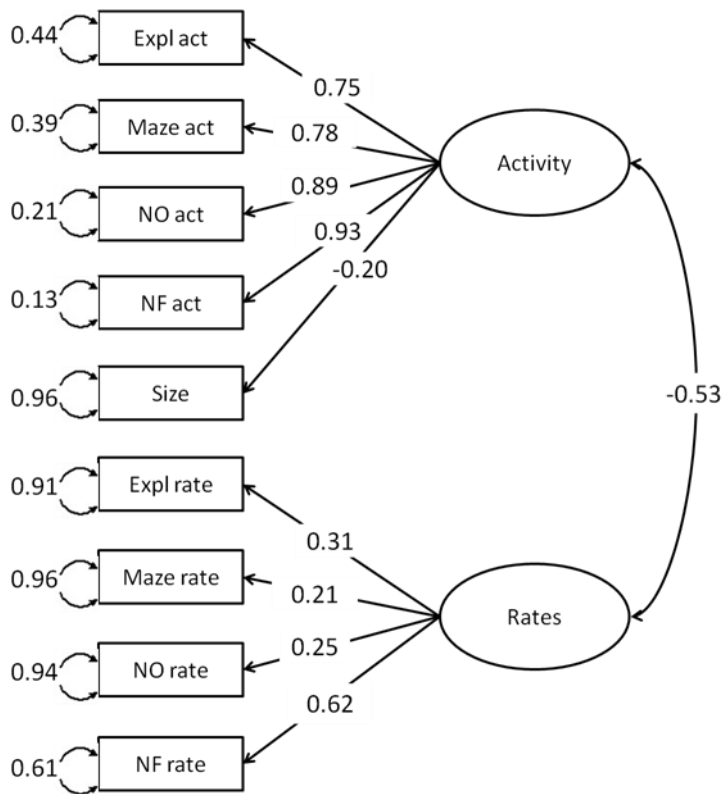


Figure 8. Model H5 (activity vs. rate) with body size included in the activity syndrome. Standardized factor loadings are shown on the one-headed arrows. The double-headed arrows shown next to each of the observed variables (squares) show the error variance that is left unexplained by the latent variable (circles), while the double-headed arrow between the latent variables show the correlation between them. Negative factor loadings for body size shows that smaller individuals are more active than larger individuals.

Discussion

We examined what have previously been termed animal personality and innovative behaviours to see how they might contribute to a wider behavioural syndrome in the guppies. By comparing different hypotheses using SEM, we found that the model including the activity measurements and rate measurements as two latent variables provided the best fit out of the seven hypotheses. The activity variables had higher factor loadings in than the rate variables, indicating that activity had a greater influence within the syndrome. This was also clear from the correlation matrix, where the correlations between the activity measurements were much higher than those between activity and rate or between the rates themselves. However, since this best model (H5 - activity vs. rates) had a significantly better fit than H6 (only activity), it is clear that co-variation between the rates also contribute the syndrome, and that they were not merely driven by the co-variance with levels of activity within the same test (H4). The co-variance between the two latent variables was negative, which means that more active individuals solved the different tasks faster, as might be expected. This could also be seen from the correlation matrix, where most of the activity variables correlated negatively with the rate variables, while the rates correlated positively with each other.

The syndrome apparent here did not consist of separate animal personality and innovation elements the way we had expected (H2), and it might be that the classification of the different behaviours in this way is an artificial construct based on research traditions (e.g. see the quite separate books on these topics by Reader and Laland 2003, Carere and Maestriperi 2013). Any behavioural measure may contain elements of personality and/or innovation, as currently defined (see Introduction). Approaching a novel object and novel food might not be any different in any fundamental way for the guppies, even though they are currently categorized by researchers as personality and innovation behaviours. Thus, the innovative behaviours we measured (i.e. the maze and novel food trials) might not necessarily have been 'innovation' behaviours at all, and might just reflect personality aspects such as boldness and shyness in exploration of novelty. However, these assays were chosen because they are similar to tests that have previously been used to measure innovation for guppies in studies by Laland and Reader (1999a), and, as mentioned in the introduction, the utilization of a novel food is the most common form of innovative behaviour (Reader and Laland 2003). Since the rate variables here constitute a single latent variable, this could be driven by individual differences in curiosity, boldness and hence tendency to innovate and might therefore represent an aspect of animal personality, with more exploratory/active individuals being more innovative, as mentioned previously. However, there was still little clear evidence that either sex or body size affected anything like the rates of innovation behaviour in our guppies.

We found no effect of population on the syndrome structure. These guppies have been in captivity for over 20 years, and they have not been under any natural selection pressure, like

predators or food limitation, for all this time. Dingemanse et al. (2007) showed that three-spine sticklebacks living in predator-free environments had weaker syndrome structures compared with sticklebacks that lived in areas with predators. Behavioural differences between wild guppy populations due to difference in predation pressure has also been shown (Harris et al. 2010). Predation pressure is the main difference between these populations, where predator fish is absent only in the Paria River (Reznick et al., 1996). The lack of any predator pressure on Campo and Quare over many generations in captivity might have reduced such differences between the populations. The univariate tests only including population showed that there were possible differences between activity levels, however, with predation-free Paria having the lowest activity level. There was also a clear difference in exploration rate, with the guppies from Paria exploring the area faster than the two other populations, perhaps as an inherited effect of their ancestors coming from a low-predation environment. Thus, guppies from the Paria River showed a more proactive approach to a novel environment by having a more bold directional movement that perhaps was lacking as part of an anti-predator strategy in the other two populations. In the study from Harris et al. (2010), guppies from a low-predation site were more cautious when emerging from a shelter than guppies from a high-predation site. This might be the same strategy that we see in our proactive individuals from the predation-free Paria River. All these effects were masked by sex and body size, however, in the population effects in the full models, suggesting possible confounding effects of state and/or life history on any such existing ancestral population effects.

We found no effect of sex on the activity syndrome (H6 – only activity), but whether or not there was any effect on the rates syndrome is unclear since the best model (H5 – activity vs. rates) did not run for the male subset. Since model H5 runs on the female subset, it might be a difference in structure between the sexes, but it is unclear in what way. Few effects of sex were found on the rate variables in the univariate tests, which might indicate that there are no differences between the sexes in the rate syndrome either. However, the univariate test only tested for the difference in mean, and not the differences in the co-variance between the variables, thus not sex differences in the syndrome structure *per se*. We did find some effects of sex on activity level in some of the univariate tests, which interestingly included females being *less* active than males, this being significantly so in the context of a novel food. This is the opposite of what Reader and Laland (1999a, b) found in their guppies, and is contrary to much of the reasoning and predictions at the beginning of this project (see Introduction). It has been found for guppies caught in the wild that females are more cautious when emerging from a shelter compared to males (Harris et al. 2010). Female fitness depends more in longevity since larger, and thus older, females produce more progeny. The females can also store sperm and do not necessarily need to seek out males to produce more broods (Reznick and Yang 1993). Males, on the other hand, constantly need to seek out new mates to increase their reproductive outcome, and thus have to take more risks. In our study, the guppies were not allowed to emerge from the shelter by their own

accord, but the lower activity level in females might reflect their more cautious nature in this new environment. Also, females spend more time in shoals than males (Magurran et al. 1992), and females in our study might therefore have been more affected by the separation from the rest of the shoal than the males were, resulting in relatively lower activity levels for females. This might explain our results here in terms of differences in personality between the sexes, but these arguments are hard to reconcile with published work on innovation in this species (Laland and Reader 1999b, a, Reader et al. 2003).

One reason for the contrasting results concerning sex differences in guppy behaviour could be the different origins and holding conditions of the fish used in different labs. In most of the univariate tests there were no effect of sex, which is contrary to the findings of Laland and Reader (1999a). This might be caused by the fact that our guppy populations have been out of their natural environment for many generations, without any selection due to predators and perhaps even competition and innovation in foraging since they were always held in the same large tanks and fed the same food *ad libitum*. However, all of this was also true for the pet-shop guppy populations used by Laland and Reader (1999b, a), but in this case our knowledge of the history of their holding conditions and the consistency of these conditions between owners is much less certain. Thus, if reproduction in female guppies is limited by availability of food resources, driving greater rates of innovation in females compared to males, it could have been due to a greater variety of environments being experienced by pet-shop populations. However, it is hard to know for sure whether this should not have been equally true for our populations that were kept in similar but perhaps more constant conditions. Therefore, it might be hard to get any clear or consistent results from different aquarium studies of different populations that have been domesticated for a longer period, since most of the naturally selective forces have been removed and/or changed, and it is hard to understand what actually is going on in these populations. This has been shown in another model species in behavioural studies, the zebra finch (*Taeniopygia guttata*), where domesticated populations were found to differ significantly in many important aspects of their behaviour from the wild population, and also from each other (Forstmeier et al. 2007). Clearly, more standardised comparisons of this type are needed for guppies, or behavioural studies need to be carried out on exclusively recently wild-caught populations or captive populations of known selective differences.

We did find a significant effect of body size on the syndrome structure when it was added as part of the activity syndrome, where smaller individuals were more active than larger individuals. Body size also had effect when added as a part of the rate syndrome, though not significantly different from the model with just the variance term. Between-individual differences in body size might therefore actually drive the whole syndrome in this study system. The univariate tests showed corresponding results, where the effect of body size was significant in the novel food trial, but showed the same trend in the other tests. This effect was only present within females, thus males were significantly more active than female not simply due to their smaller size compared with females. All of these effects were

most obvious in the novel food trial, which was the only trial that involved food being present, clearly suggesting a foraging context for these differences. Size is expected to be an important factor for female fecundity (Reznick and Yang 1993), which might cause smaller females to search more for food and thus be more active than both males and larger females. Activity level might therefore be driven by the developmental state of the individual, since size and age is highly correlated within the guppies (Reznick et al. 1996). The behaviours we measured might therefore not show animal personalities, as such, but rather just be an effect of the individual developmental state, where smaller fish need to search more for new sources of food either due to more energy needed for growth or lower competitive ability around already discovered foods. To show that the behaviours reflect animal personalities, repeated measures should have been taken in a different developmental state to show individual consistency over a longer time period. In this way, we could also have looked at whether difference in growth would have affected the syndrome structure, i.e. a pace-of-life syndrome, where individuals with a more rapid growth takes more risks in the form of exploration and innovation, thus live fast and die young (Reale et al. 2010b).

This study was specifically designed to maximize the sample sizes for between-subjects comparisons concerning personality and innovation behaviours, and specifically to explore sex, size and population differences in behaviour. It was therefore decided to maximize the sample sizes in the between-individual comparisons by not carrying out any repeated sampling within individuals. Repeated measures of the behaviours within the same individuals would perhaps have provided more insight into the levels of long-term consistency in these behaviours in distinguishing the strength of any animal personality (Nicolaus et al. 2012, Wolak et al. 2012). We did have repeated measures for the level of activity, and the high individual repeatability clearly shows that the guppies show individual personality when it comes to activity, at least within the short-term of these trials. However, the measurements were taken during a short time interval and it could therefore be argued that they do not reflect true repeated measures (Wolak et al. 2012).

It is also clear from the high correlations between the tests that activity levels were not situation specific, but showed comparable differences over time and tests. From the habituation model it was shown that the activity never dropped down to a base-line level. In addition, the interaction between time and the four different tests was not significant, indicating that the slope was not significantly different between the four tests, thus it did not flatten out. The fish were, however, in the process of habituation, seen by the significantly negative effect on activity over time (Peeke et al. 1971). In addition, size affected the rate at which the guppies habituated, with larger individuals habituating faster than smaller individuals. Thus, the higher activity level for smaller individuals is probably an effect of the slower habituation rate compared to larger individuals. If we had more time for further analyses, it would be interesting to see if the rate of habituation showed individual

consistency, and to add this into the SEM models to see how they would affect the behavioural syndrome.

In studies of animal personalities and behaviour syndromes, it has become clear that large sample sizes are needed, not only by increasing the number of individuals, but also by having larger numbers of repeated measures for each individual (Wolak et al. 2012). This increases the chance of detecting personalities within individuals, and also reduces the variance between individuals when investigating behavioural syndromes. In addition, it opens up the possibility for exploration of within- and between-individual co-variance between multiple variables simultaneously (Araya-Ajoy and Dingemanse 2014). Extensive sample sizes also make it possible to include a genetic pedigree, and to test for quantitative genetic effects on both individual levels of behaviour (personality) and behavioural co-variances (syndromes) – see Dingemanse and Dochtermann (2013).

Research in animal personalities and behaviour syndromes is still relatively new, and most studies still merely attempt to detect if there is any personality or syndrome present. However, as the research field matures, it becomes more interesting to study how any individual variance are created and maintained within natural populations, and to see how selection works on different personalities and behavioural syndromes. To understand the biological and a-biological processes that affect syndrome structures, we have to test *a priori* hypotheses concerning trait co-variation and correlated selection within a theoretical framework (Armbruster et al. 2014). The SEM approach taken here allows us to make predictions concerning the structure of trait co-variances, and to compare these hypothesized structures with each other (Dochtermann and Jenkins 2007). In this way, we can also predict how the structure might change under different selection pressures, both between populations within the same species, and between different species. This approach also makes it possible to look at the strength and directions of the different co-variances within sub-groups within the data set at the same time. SEM is therefore suggested as the statistical method of choice when analysing behavioural syndromes (Dingemanse et al. 2010a). A weakness with this method is that it is affected by the strength of the co-variances. In this case, the best model (H5 – activity vs. rate) did not run for the male subset, presumably due to low co-variances between the rates.

In this study, a PCA was used half-way through the SEM analysis in order to get a better idea of how the different behaviours co-varied, since the first SEM models (H0-H3) did not seem to tell the whole story or sufficiently test the data. However, when comparing models in SEM, this kind of use of PCA's should generally to be avoided. This is because PCA is a theory-free approach that is applied merely to see how the different variables co-vary (e.g. in data reduction procedures). It does not explain why they co-vary, and the unique nature of the results per data set often makes them hard to compare with other studies (Budaev 2010). Another important aspect when moving forward towards comparative studies is to measure clearly defined characters, and to standardize the way they are measured. In this

way the same characters can be measured for different populations and/or species, and the co-variances can be compared properly. As mentioned in the introduction, several studies have used the same and different kinds of tests to measure exploration and innovation, so this field clearly needs a more defined methodology, especially for work within the same species.

Conclusion

Within the guppies, body size had an effect on the syndrome structure, and developmental state might thus drive the whole syndrome recorded here. This is also clear from the habituation model, where larger individuals were shown to habituate faster than smaller individuals, which also explains why smaller individuals have a higher activity level. To get a better understanding of how developmental state affects activity level in the future, repeated measures in a different developmental state should be provided. This could also be used to see if there is any developmental state where innovative behaviours are more important, either because more food resources are needed due to growth (or reproduction), or just because smaller, younger individuals have lower competitive abilities. By following individuals over a longer time period it would also be possible to see differences due to early life experience. For instance how food availability during early development may affect the syndrome structure, and could confirm if the results here are part of a wider pace-of-life syndrome.

The lack of any difference between both sex and population in behaviours where they have been shown to differ in earlier studies indicates that it is hard to get consistent results when comparing between domesticated populations. In addition, most of the natural selection pressure is weaker or removed entirely, and it might be hard to find any answers to predictions based on the ecology in their wild habitat. Thus, experiments like this should preferably be conducted on wild-caught populations, where the natural selection pressure is kept intact, tested in a laboratorial set-up where any confounding effects can be controlled for and sufficiently large sample sizes can allow appropriate statistical analysis.

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Appendix A

A mixed effect model was used to test whether the guppies habituated, and if either sex or body size affected this habituation. The model included test, time, sex and body size as fixed effects, and fish identity as a random effect. Since the full model had a lot of non-significant interactions, model reduction was carried out using a comparison of p-values and AIC values (see Table A.10 and A.11). This way, the number of variables is reduced, and there is less chance for any significant effects dropping out only due to chance. However, by selecting only significant effects, the estimates of these effects tend to be biased upwards, called the ‘winners curse’ (see Forstmeier & Schielzeth 2011). To try and avoid this problem, we only accepted model simplifications that reduced the overall AIC values of the models, and thus kept all the two-way interactions, even though not all of them had a significant effect (see Table 5). Model 4 had the lowest AIC value, and was therefore presented in this study as the final model. All models were run with and without the maze, with similar results.

Table A.10. Comparison of the habituation models, both with and without the maze. The full model (Model 1) contains all interactions; Model 2 contains all the two – and three-way interactions; Model 3 contains all the two-way interactions in addition to the three-way interaction between time, size and sex, since this was shown to be significant in Model 2 ($F_{1,1229} = 5.26$, $p = 0.02$); and Model 4 contains only the two-way interactions (see text for more information). The three-way interaction between time, test and size was not significant in Model 3 ($F_{1,1238} = 2.46$, $p = 0.12$). Due to parsimony, Model 4 was presented in this study (see Table 5 in the main text). Activity was square root transformed, and fish identity is added as a random effect in all models. K is the number of parameters estimated.

Model	With maze			Without maze		
	K	AIC	Δ AIC	K	AIC	Δ AIC
Model 4	21	5796.89	0.00	17	4487.36	0.00
Model 3	22	5803.19	6.30	18	4494.38	7.02
Model 2	31	5831.00	24.11	24	4507.74	20.38
Model 1	34	5838.19	41.30	26	4512.40	25.04

Table A.11. Results from the full habituation model with test, time, size and sex and all the interactions as fixed effects, and fish identity as a random effect. Activity was square root transformed. The degrees of freedom were calculated with a Satterthwaite approximation, and both the effect degrees of freedom and the error degrees of freedom are given in each case. Highly significant effects ($p < 0.01$) are in bold, while moderately significant effects ($p < 0.05$) are in frames. Most of the moderately significant effects of the three-way interactions disappeared when the four-way interaction was removed. The sample size was 1368 for the models including the maze and 1026 for the model without the maze.

Variables	With maze			Without maze		
	df	F	p	df	F	p
Test	3, 1226	5.48	0.03	2, 892	1.06	0.35
Time	1, 1226	23.85	<0.001	1, 892	19.99	<0.001
Size	1, 1291	5.15	0.02	1, 1001	4.16	0.04
Sex	1, 1291	0.08	0.77	1, 1001	0.26	0.61
Test:Time	3, 1226	0.40	0.81	2, 892	3.62	0.03
Test:Size	3, 1226	0.48	0.75	2, 892	0.86	0.42
Test:Sex	3, 1226	0.51	0.67	2, 892	5.44	0.02
Time:Size	1, 1226	17.98	<0.001	1, 892	15.85	<0.001
Time:Sex	1, 1226	3.29	0.04	1, 892	5.44	0.02
Size:Sex	1, 1260	0.81	0.44	1, 1001	0.32	0.57
Test:Time:Size	3, 1226	2.60	0.05	2, 892	3.17	0.04
Test:Time:Sex	3, 1226	0.04	0.84	2, 892	1.24	0.29
Test:Size:Sex	3, 1226	0.001	0.98	2, 892	0.34	0.71
Time:Size:Sex	1, 1226	5.27	0.02	1, 892	6.31	0.01
Test:Time:Size:sex	3, 1226	1.64	0.18	2, 892	1.52	0.22

Appendix B

PCAs were conducted to investigate the co-variance structure between the eight behaviour variables. These were done without a rotation (Table A.12) and with a varimax rotation (Table A.13). In the un-rotated PCA, all the activity variables in addition to rate during the novel food test dropped out in the first principal component (PC), while all the other rates dropped out in PC2. These were the only significant PC's with eigenvalue 3.33 and 1.38 respectively. In the varimax rotated PCA, all the activity measurements dropped out as PC1, while the rates dropped out singularly as PC2-PC5. Only the first PC was significant though, with an eigenvalue of 1.97.

Tabell A.12. Results from the un- rotation PCA. Eigenvalues and the proportion of the variance explained are given for all the PCs, in addition to the loadings for each variable within these PCs. Strong loadings (> 0.30) are shown in bold.

	PC1	PC2	PC3
Eigenvalue	3.33	1.38	0.96
Proportion of variance	0.42	0.17	0.12
Cumulative variance	0.42	0.59	0.71
expl_act	0.84	0.05	0.05
expl_rate	-0.18	0.58	0.61
maze_act	0.83	0.18	0.24
maze_rate	-0.14	-0.71	-0.38
no_act	0.89	0.15	-0.02
no_rate	-0.18	0.65	-0.37
nf_act	0.91	0.12	0.01
nf_rate	-0.49	0.23	0.49

Table A.13. Results from the PCA with a varimax rotation. Eigenvalues and the proportion of the variance explained are given for all the PCs, in addition to the loadings for each variable within these PCs. Strong loadings (> 0.30) are shown in bold.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	1.97	1.03	1.01	1.01	1.00
Proportion of variance	0.25	0.13	0.13	0.13	0.13
Cumulative variance	0.25	0.38	0.51	0.64	0.77
expl_act	0.42	-0.15	-0.04	-0.04	-0.05
expl_rate	-0.05	0.08	0.09	0.99	0.05
maze_act	0.49	-0.07	-0.09	0.04	0.01
maze_rate	0.01	0.03	0.98	0.09	0.13
no_act	0.91	-0.16	0.05	-0.05	-0.06
no_rate	-0.05	0.05	0.13	0.05	0.99
nf_act	0.83	-0.15	-0.03	-0.06	-0.02
nf_rate	-0.17	0.97	0.03	0.09	0.05

Appendix C

Separate correlation matrixes was carried out for each sex to see if there were any clear differences between them, which could explain why model H5 (activity vs. rates) would not run on the subset for the males. As can be seen, some of the correlations with rates are lower for males (Table A.14) than for females (Table A.15), which might explain why the model collapsed on the male subset.

Table A.14. Pair-wise correlations between the 8 behavioural variables for males. The significant correlations are marked in bold, while correlations within the same tests are in frames.

	Exploration		Maze		Novel object		Novel food	
	Activity	Rate	Activity	Rate	Activity	Rate	Activity	Rate
Exploration activity	1	$r=-0.01$ $p=0.93$	$r=0.70$ $p<0.001$	$r=-0.02$ $p=0.84$	$r=0.66$ $p<0.001$	$r=-0.23$ $p=0.01$	$r=0.72$ $p<0.001$	$r=-0.33$ $p<0.001$
Exploration rate		1	$r=0.10$ $p=0.27$	$r=0.15$ $p=0.10$	$r=-0.04$ $p=0.69$	$r=-0.02$ $p=0.83$	$r=-0.09$ $p=0.33$	$r=0.16$ $p=0.09$
Maze activity			1	$r=0.04$ $p=0.62$	$r=0.71$ $p<0.001$	$r=-0.04$ $p=0.71$	$r=0.79$ $p<0.001$	$r=-0.20$ $p=0.04$
Maze rate				1	$r=0.16$ $p=0.09$	$r=0.36$ $p<0.001$	$r=0.04$ $p=0.69$	$r=0.01$ $p=0.89$
Novel object activity					1	$r=-0.13$ $p=0.18$	$r=0.83$ $p<0.001$	$r=-0.36$ $p<0.001$
Novel object rate						1	$r=-0.07$ $p=0.48$	$r=0.39$ $p<0.001$
Novel food activity							1	$r=-0.33$ $p<0.001$
Novel food rate								1

Table A.15. Pair-wise correlations between the 8 behavioural variables for females. The significant correlations are marked in bold, while correlations within the same tests are in frames.

	Exploration		Maze		Novel object		Novel food	
	Activity	Rate	Activity	Rate	Activity	Rate	Activity	Rate
Exploration activity	1	r=-0.15 p=0.12	r=0.56 p<0.001	r=-0.27 p=0.004	r=0.63 p<0.001	r=0.05 p=0.61	r=0.61 p<0.001	r=-0.33 p<0.001
Exploration rate		1	r=-0.07 p=0.47	r=0.29 p=0.002	r=-0.16 p=0.09	r=0.24 p=0.01	r=-0.13 p=0.18	r=0.20 p=0.03
Maze activity			1	r=-0.52 p<0.001	r=0.64 p<0.001	r=-0.02 p=0.85	r=0.64 p<0.001	r=-0.24 p=0.01
Maze rate				1	r=-0.27 p=0.004	r=0.13 p=0.16	r=-0.27 p=0.003	r=0.19 p=0.04
Novel object activity					1	r=-0.10 p=0.30	r=0.83 p<0.001	r=-0.33 p<0.001
Novel object rate						1	r=-0.09 p=0.34	r=-0.16 p=0.10
Novel food activity							1	r=-0.35 p<0.001
Novel food rate								1