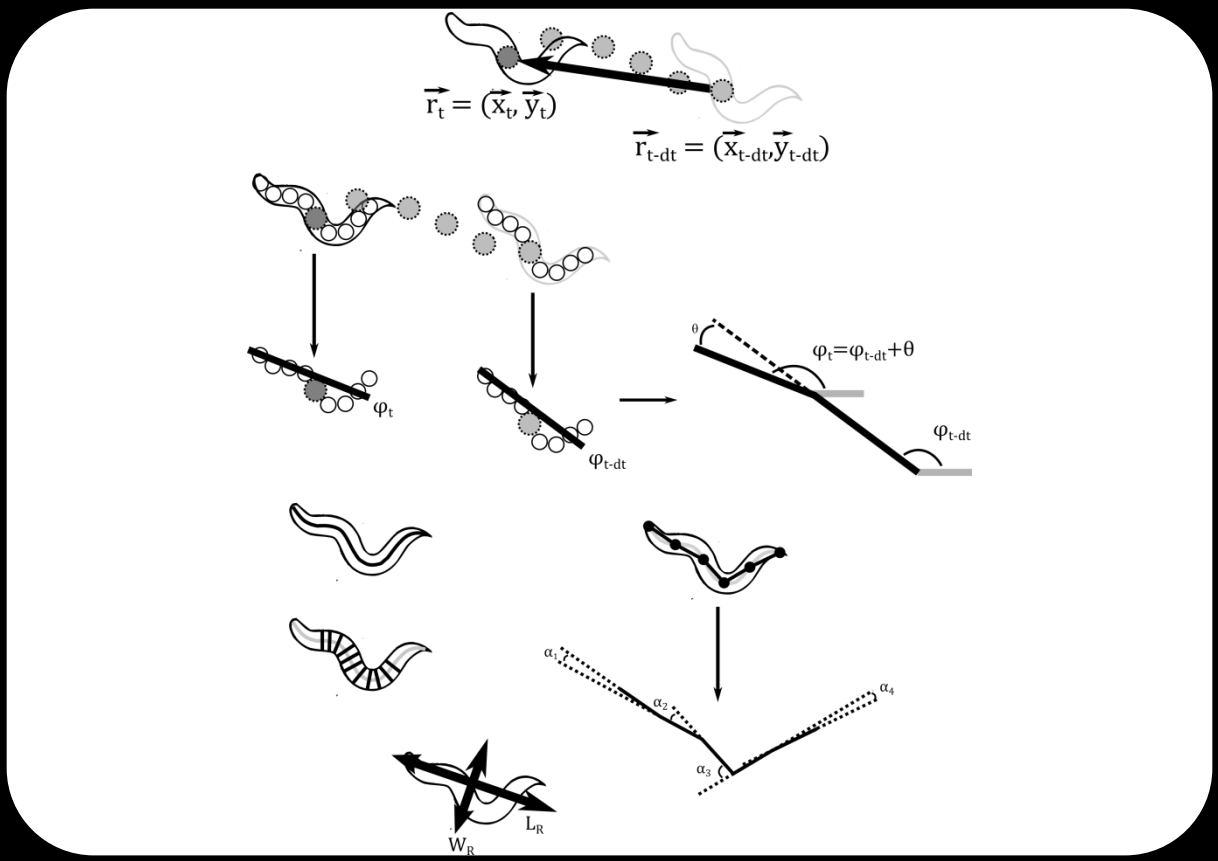
Natural selection and evolution of behaviour and its variability in experimental populations

Bruno Alexandre Caetano Afonso



Dissertation presented to obtain the Ph.D degree in Evolutionary Biology

Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

Oeiras,
August 2016

UNIVERSIDADE NOVA DE LISBOA

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Summary

Behaviour has evolved in animal species as an output from the function of energetically expensive nervous systems. Notwithstanding, the ability to perform behaviour seems to have persisted in animal species until today, hence behaviour in general might have a significant adaptive value. Such value has been demonstrated experimentally in some instances.

However, in the latest years, it has been hypothesized that not only the performance of a given behaviour, but also the variability of behaviours performed can have an adaptive value. On one hand, variability in behaviour is usually high when an animal attempts to learn a skill – for it explores different possible behaviours – and decreases throughout learning – for the animal then exploits the actions most reinforced. On another hand, behaviour variability can be both increased and reduced through specific neural activity. Last, but not least, it has also been shown in experimental populations that learning can be adaptive.

Since natural environments are generally ever-changing and unpredictable, it seems reasonable to presume that an animal might need to learn novel actions quite frequently in order to have more chances of reproducing. This frequent novel action learning could imply frequent exploration of different behaviours, which would imply frequent generation of behaviour variability. Consistently with this idea, it has been found that individuals with higher behaviour variability show higher learning rates. However, the adaptive value of behaviour variability had never been assessed experimentally.

In this thesis, I have measured several behavioural features – ranging from postural to locomotor – using the Multi-Worm Tracker (MWT) in experimental populations of *Caenorhabditis elegans* to assess whether such adaptive value could really exist. These populations – one ancestral

and three derived – were involved in a 50-generation experimental evolution in a changing environment, in which NaCl concentration of Petri dishes has progressively increased from 25 to 305 mM from generations 0 to 35 and maintained in 305 mM between generations 35 and 50. On one hand, this progressive increase allowed a constant environmental change that would eventually trigger the need to frequently explore novel actions that could help facing the environmental stress caused by the increasing osmotic pressure. On the other hand, a progressive NaCl concentration increase could mitigate selection pressures at the physiological level because *C. elegans* can physiologically acclimate to increasing NaCl concentrations if they are imposed gradually. By using different summary statistics of the behavioural features tracked, one can distinguish between specific behaviours being performed and the variability of behaviours being performed. The first mode of behaviour is captured by a location statistic; here, the median along tracking time of each individual nematode tracked is used and this mode of behaviour was termed as behaviour centrality. The second mode of behaviour was termed as behaviour variability and was captured using a scale statistic, here the median absolute deviation from the median (MAD) along tracking time. Behaviour was always quantified in the environment in which experimental evolution has begun – NGM with 25 mM NaCl – and in the environment in which experimental evolution has ended – NGM with 305 mM NaCl.

The adaptive value of behaviour and its variability was assessed by estimating approximations of natural selection surfaces on the ancestral population in both the aforementioned environments. For these estimations, fecundity and behaviour were quantified in inbred lines derived from the ancestral population. The univariate quadratic approximations of the selection surface – those estimated using one behavioural feature at a time – have shown widespread directional and

stabilizing selection on behaviour and its variability. The directional selection was highly congruent with the evolutionary responses observed. However, the univariate selection surfaces do not distinguish between direct selection on a behavioural feature and indirect selection on that feature, due to correlation to another one. In an attempt to distinguish these, selection coefficients of several behavioural features – including both behaviour centrality and variability features – were simultaneously estimated through multivariate approximations of the selection surfaces. Yet, when these multivariate selection surfaces were taken into account, direction of selection was opposite to the evolutionary responses observed in many features. Also, the regressions used to estimate the multivariate selection surfaces were highly multicollinear, rendering the obtained estimates unstable and very susceptible to changes depending on the features included in the regression. To circumvent this multicollinearity, approximations of the multivariate selection surfaces were estimated using principal components of both behaviour centrality and variability altogether. In these, multicollinearity was still present, but in a lower degree and evolutionary responses were again little congruent with the selection coefficients there estimated, for most of the feature principal components were under selection but have not evolved.

These results suggest that even if there was direct selection favouring evolution of behaviour and its variability, such a direct selection seemed to be overruled by a stronger, indirect selection, due to correlation to unmeasured phenotypes, which has shaped the observed evolutionary response. Therefore, an adaptive value of behaviour variability cannot be neither endorsed, nor excluded, but does not seem likely under this experimental setting. For a direct demonstration or exclusion of this theory, learning rate of inbred lines with different behaviour variabilities should also be assessed, ideally by performing an operant learning task.

Resumo

O comportamento evoluiu nas espécies animais como resultado da função de sistemas nervosos dispendiosos a nível energético. Não obstante, a capacidade de executar um determinado comportamento parece ter persistido nas espécies animais até à actualidade, o que sugere um valor adaptativo significativo para o comportamento em geral. Tal valor foi demonstrado experimentalmente em vários casos.

No entanto, nos últimos anos tem sido colocada a hipótese de que não só a execução de um dado comportamento, mas também a variabilidade no espectro de comportamentos manifestados pode ter um valor adaptativo em si mesma. Por um lado, a variabilidade no comportamento é elevada quando um animal está a aprender algo novo – porque este explora vários comportamentos possíveis – e diminui à medida que o animal avança no processo de aprendizagem – porque o animal vai restringindo o seu leque de comportamentos àqueles que são mais reforçados ao longo da aprendizagem. Por outro lado, a variabilidade no comportamento pode aumentar e diminuir como consequência de actividade neuronal específica. Por último, mas não menos importante, foi também demonstrado em populações experimentais que a aprendizagem pode ser adaptativa.

Uma vez que os ambientes naturais podem alterar-se com frequência e de forma imprevisível, parece razoável presumir que um animal necessite constantemente de aprender novas acções de forma a ter mais hipóteses de se reproduzir. Esta aprendizagem frequente de novas acções implicaria a exploração constante de novos comportamentos, o que implicaria a geração de variabilidade no comportamento. Em concordância com esta ideia, foi também demonstrado que indivíduos que manifestam maior variabilidade no comportamento mostram

também uma maior taxa de aprendizagem. Porém, o valor adaptativo da variabilidade no comportamento nunca foi testado experimentalmente.

Nesta tese, medi várias características comportamentais – desde características posturais a locomotoras – usando o *Multi-Worm Tracker* (MWT) em populações experimentais de *Caenorhabditis elegans* para testar a existência de um valor adaptativo para a variabilidade no comportamento. Estas populações – uma ancestral e três derivadas – estiveram envolvidas numa evolução experimental de 50 gerações num ambiente em alteração, no qual a concentração de NaCl nas placas de Petri foi aumentando de 25 mM para 305 mM desde a geração 0 à 35, tendo sido mantida em 305 mM entre as gerações 35 e 50. Por um lado, o aumento progressivo da concentração de NaCl permite um alteração ambiental constante que poderia desencadear a necessidade frequente de explorar novas acções que poderiam ajudar a lidar com o stress ambiental causado pelo aumento da pressão osmótica do meio. Por outro lado, uma vez que *C. elegans* pode aclimatar-se a nível fisiológico ao aumento da concentração de NaCl se tal for imposto gradualmente, uma possível pressão selectiva ao nível fisiológico poderia ser atenuada. Usando diferentes estatísticas sumárias das características comportamentais quantificadas, podemos distinguir entre a execução de comportamentos específicos e a variabilidade nos comportamentos executados. O primeiro modo de comportamento é descrito por estatística de localização; neste caso, foi usada a mediana dos valores obtidos ao longo da monitorização de cada indivíduo e este modo comportamental foi designado por centralidade do comportamento. O segundo modo de comportamento foi designado por variabilidade do comportamento e descrito por uma estatística de escala, no caso o desvio absoluto mediano em relação à mediana dos valores de cada característica ao longo do tempo de monitorização. O comportamento foi sempre quantificado no ambiente no qual evolução experimental se

iniciou – NGM com NaCl a 25 mM – e no ambiente em que a evolução experimental terminou – NGM com NaCl a 305 mM. O valor adaptativo do comportamento e da sua variabilidade foi avaliado estimando aproximações das superfícies de selecção natural sobre população ancestral em ambos os ambientes mencionados. Para estas estimações, foram quantificados o comportamento e a fecundidade de linhas endogâmicas derivadas da população ancestral. As aproximações quadráticas das superfícies de selecção univariadas – estimadas usando uma característica comportamental de cada vez – mostraram uma ampla selecção direccional e estabilizadora no comportamento e na sua variabilidade. A selecção direccional, por sua vez, foi altamente congruente com a resposta evolutiva observada. Porém, as superfícies univariadas de selecção não distinguem entre selecção directa numa característica comportamental e a selecção indirecta nessa característica, devido à correlação desta com outra característica. Numa tentativa de fazer esta distinção, foram estimados coeficientes de selecção em várias características comportamentais em simultâneo – incluindo características de centralidade e variabilidade do comportamento – por aproximações das superfícies de selecção multivariadas. No entanto, em muitas das características a direcção sugerida pela selecção tendo em conta as superfícies de selecção multivariadas era oposta à resposta evolutiva observada. Além do mais, as regressões usadas para estimar as superfícies de selecção multivariadas eram em larga medida multicolineares, o que leva a que as estimativas obtidas sejam instáveis e susceptíveis a alteração drástica dependendo do conjunto de características incluídas na regressão. Com o intuito de resolver a multicolinearidade, foram estimadas aproximações das superfícies de selecção multivariadas usando componentes principais da centralidade e variabilidade do comportamento em conjunto. Nestas superfícies de selecção, a multicolinearidade ainda estava presente, embora em grau

muito menor, e a resposta evolutiva destes componentes também era pouco congruente com a direcção sugerida pela selecção, uma vez que a maioria destes componentes estava sob selecção mas não mostrou resposta evolutiva.

Estes resultados sugerem que mesmo tendo existido selecção directa a favorecer a evolução do comportamento e da sua variabilidade, tal selecção foi contraposta por uma selecção mais forte e indirecta, devido à correlação com características não quantificadas nesta tese; esta selecção indirecta, por sua vez, terá sido a selecção que comandou a resposta evolutiva observada. Deste modo, um valor adaptativo para a variabilidade no comportamento não pode ser suportado nem excluído por estes dados, mas parece pouco provável neste contexto experimental. Para demonstrar ou excluir esta possibilidade directamente, a taxa de aprendizagem destas linhas endogâmicas da população ancestral deverá também ser quantificada, idealmente através da execução de uma tarefa que envolva um condicionamento operante.

Chapter 1: Introduction

We have always been amazed with the diversity of behaviour in the living world. From the locomotion in many forms – swimming, crawling, walking, running –, passing through strategies for parental care, hunting, escaping, up to aggressive behaviour in competition for resources and sexual partners, many are the forms by which living organisms behave. The concept of behaviour when applied to life seems very intuitive to any of us, yet it is still controversial at the scientific level (Levitis et al., 2009). What is behaviour? We can say it is an action that involves the physical motion of a living organism and performed under a given context that is the surrounding environment. We should recognize, however, that this phenomenon is widely observed among living systems. Many bacteria, for instance, are able to move their single-cell bodies according to their chemical environment through the rotation of long appendages called flagella (Wadhams and Armitage, 2004). Single-cell (Glaser, 1924) and colonial protists (Holmes, 1903) are also able to locomote through small appendages called cilia, which are widely dispersed throughout their cells. Sponges crawl along the soil at speeds up to 4 mm/day through propagation of cell contraction waves (Bond and Harris, 1988) and show periodic and extensive overall body contractions (Nickel, 2004), even though they are so simple animals as they have no tissue organization (Hickman Jr. et al., 2011). Even plants, such as *Mimosa pudica*, show leaf movements in response to mechanic stimuli (Weintraub, 1952).

In the context of this thesis, I define behaviour as the motion of a living organism's body produced by forces generated inside that same organism. This motion can involve the entire body or only body parts, as long as these remain integrated in the whole organism. Behaviour is a property of an individual organism and not of a body part; even when behaviour only involves a single body part of a given organism; the agent

performing behaviour is the organism, not the body part. As an example, many vertebrate species shed a body part – usually a limb or the tail – as an extreme strategy to escape from predators, a phenomenon termed as autotomy. In many cases, that body part is able to move after being shed from the remainder of the body (Higham et al., 2013), but that body part is not behaving, according to the definition used in this thesis. The absence of motion can also be considered as behaviour, but only if there are no physical constraints for that motion to occur. For instance, rats react defensively to the presence of potential predators by assuming an immobile posture termed as freezing, which has been interpreted as a fear response (Blanchard and Blanchard, 1971). Under this definition, freezing should be considered as a behavioural response, because there is no physical constraint for their movement to occur, but rats engage in an inaction period that is considered as an action by itself. I shall recognize that this definition of behaviour is rather incomplete, for it does not include phenomena such as skin colour change in cephalopods, which allows both camouflage within the surrounding environment and also communication among conspecifics (Messenger, 2001). Nevertheless, this definition will suffice for the sake of this thesis and shares similarities with recently published definitions. These latest definitions also emphasize that behaviour is an individual property generated inside the organism (Levitis et al., 2009) and inseparable from its external and internal contexts (Gomez-Marin et al., 2014).

In most animals, behaviour is produced as a result of the activity of a nervous system, in which neurons have a pivotal role. Neurons are cells that propagate electric signals generated by ion fluxes through their cell membranes. This whole process demands energy in the form of electric potential difference. This potential difference is generated by the differential permeability of neuron membranes to ions, on one hand, and by ionic gradients generated alongside the neuron membrane, on the

other hand. In a regular resting state of a neuron, the gradients generated are mainly constituted by an excess of potassium ions (K^+) in the internal side of the membrane and an excess of sodium ions (Na^+) on the external side. Neuron membranes have specific ion channels, through which K^+ and Na^+ flow by diffusion. In the resting state, neurons maintain an ionic gradient by actively importing K^+ and exporting Na^+ through a Na^+/K^+ pump. It is this active import and export process through the Na^+/K^+ pump associated with the leakage through diffusion of these ions in the reverse direction that generates the voltage alongside the neuron membrane. Every time a neuron is activated and an impulse is generated, the accumulated energy is spent, ion channels are opened in large numbers, ions will diffuse heavily, cancelling the gradient generated at rest and the potential difference is reversed; this process propagates as a wave throughout the membrane of each neuron. After that propagation, the potential difference has to be rebuilt at the membrane (Kandel et al., 2012). This voltage management in neurons requires a very high amount of energy (Attwell and Laughlin, 2001; Du et al., 2008). Consequently, nervous systems are very expensive. The adult human brain, for instance, represents 2% of the body mass, yet it is responsible for around 20% of total body energy expenditure (Kety, 1957; Sokoloff, 1960).

It is marvellous to realize that, notwithstanding paramount energy expenditure, the overwhelming majority of animal species have a nervous system, with a complexity that ranges from the diffuse systems of cnidarians to the highly structured systems in mammals (Hickman Jr. et al., 2011). It has been recognized also that a major function of the nervous system is to generate behaviour (Simmons and Young, 2010). The pervasive evolution of nervous systems can be considered a very significant landmark in the evolution of animal species and suggests that throughout evolution of animal species animals investing energy in an expensive nervous system might have reproduced more than animals

that refrain from such an investiment. This advantage is believed to hold in present times and therefore it is generally believed that behaviour has a significant adaptive value (Tinbergen, 1963).

Variability in behaviour and its sources

Animal behaviour can vary due to a multitude of factors, from motivational states and prior experiences to the variable nature of external stimuli (Renart and Machens, 2014). However, even when these factors seem experimentally controlled and an animal senses well-defined stimuli, behavioural responses can be highly variable (Fiske and Rice, 1955). This within-individual variation in behavioural output is known as behaviour variability[1] (Renart and Machens, 2014).

This variabilty occurs due to two main reasons. On one hand, the nervous system is inherently noisy, as any other biological system involving a relatively low number of molecules (Schrödinger, 1944; Katz and Miledi 1950, 1951; Elowitz et al. 2002; Faisal et al. 2008); this noise arises mostly from random fluctuations of molecular activity and position (Elowitz et al., 2002). On the other hand, individual animals seem able to actively change levels of behaviour variability in a more deterministic manner, by activating specific neural circuits. An anterior brain region called the lateral magnocellular nucleus of the nidopallium (LMAN) was

[1] In this thesis I use the concept of variability as used in neuroscience, which would be equivalent to variation in an evolutionary biology perspective. In evolutionary biology terms, variability is seen as the potential or propensity to display variation (Wagner and Altenberg, 1996). Here I assume that, in evolutionary biology terms, the variation observed is positively correlated with the variability as a potential. In other words, I assume that the more variation observed in behaviour of an animal, the higher the potential that animal has to generate variation in behaviour, hence the higher the behaviour variability of that animal.

found to be crucial in generating variability in zebra finch male song (Ölveczky et al., 2005; Figure 1.1), allowing the finches to produce song trials, which are essential for song learning (Kao et al., 2005; Tumer and Brainard, 2007). In mammals, circuits lying in the interface between the cerebral cortex and the basal ganglia are hypothesized to be involved in the generation of behaviour variability (Costa, 2011), yet no neural circuits have been found to generate behaviour variability.

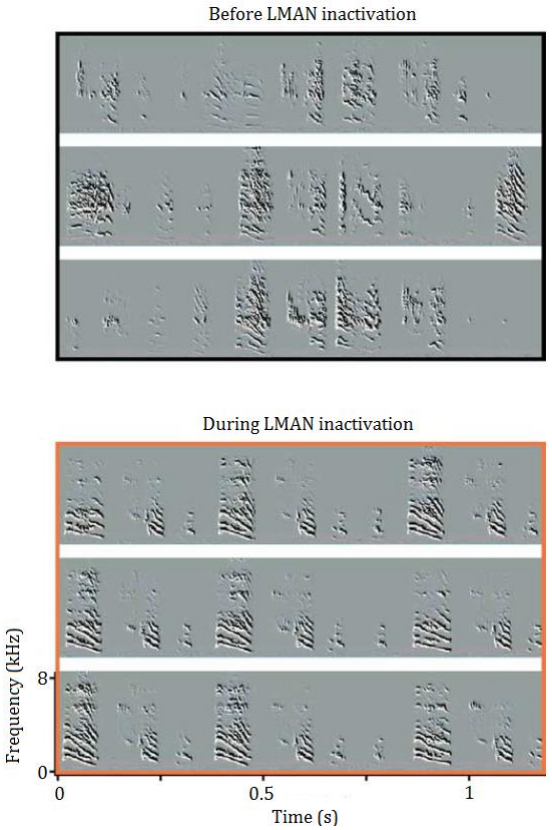


Figure 1.1 – Juvenile zebra finch song sequence and frequency lose variability and become stereotyped when LMAN is inactive. The figure shows an example zebra finch song before and during LMAN inactivation. Adapted from Ölveczky et al., 2005.

Nonetheless, it has been found in mammals that the amount of behaviour variability before learning predicts learning rate of a given task in such a way that animals initially having a higher behaviour variability learn faster (Wu et al., 2014). that reinforcement decreases behaviour variability (Jin and Costa, 2010; Santos et al., 2015; Takikawa et al., 2002) and punishment increases it (Galea et al., 2013). In the nematode *C. elegans*, dopaminergic activity through D2-like receptors has been found to decrease locomotion speed variability (Omura et al., 2012; Figure 1.2). Furthermore, it has also been found in *C. elegans* that RIM interneuron increases variability in neural activity of AIB, AVA and RIM itself in response to odours and also introduces variability in behaviour responses to those odours (Gordus et al., 2015).

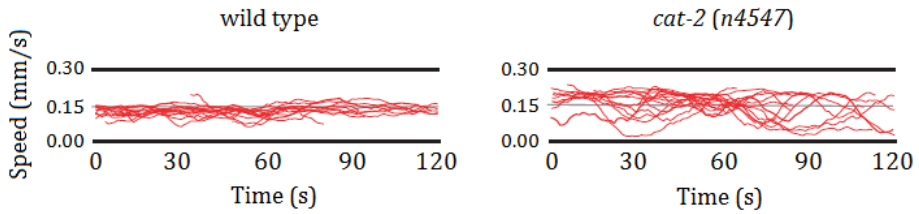


Figure 1.2 – Speed bouts from several individual *C. elegans* nematodes showing speed variability is increased in a tyrosine hydrolylase deletion mutant compared to wild-type nematodes. Tyrosine hydroxylase is the enzyme that catalyses the conversion of L-tyrosine to L-DOPA, the immediate precursor of dopamine (Chase and Koelle, 2007). Adapted from Omura et al., 2012.

These findings clearly indicate that behaviour variability is not only the outcome of stochastic noise associated to general physical and chemical phenomena involving all molecules, but it is also regulated by specific physiological processes in a deterministic manner.

The adaptive value of behaviour variation

It seems reasonable to point a high adaptive value to behaviour in general because the nervous system underlying it is very expensive. Direct demonstrations of natural selection acting on behaviour are scarce, but quite significant. Mosebach-Pukowski has provided one of the first, by showing that *Vanessa* caterpillar crowding protects them from insectivorous songbirds as isolated caterpillars are eaten more readily than those in a cluster (Mosebach-Pukowski, 1937). In another example, male sticklebacks fan their eggs by constantly swimming in their nest spots; prevention of fanning kills the eggs and artificial ventilation rescues them (Tinbergen, 1951). Seido Ohnishi found that larval feeding rate of both *Drosophila melanogaster* and *Drosophila simulans* are positively correlated with their egg-to-adult viability (Ohnishi, 1979).

On the other hand, behaviour variability has been contextualized in the exploration-exploitation trade-off. Animals tend to explore through a range of actions in order to reach actions that lead to high amount of reward in the long run – *i.e.* high value (Sutton and Barto, 1998) – and this exploration implies an increase in behaviour variability. In contrast, after an animal finds an action or set of actions that have high value, it will tend to exploit them by executing them more frequently (Sutton and Barto, 1998) and as a result behaviour variability will likely decrease. As the surrounding environment is mostly unknown by an individual animal and the animal cannot sample the value of all possible actions – as a virtually infinite number of actions are possible –, there is a constant need for a behavioural decision between exploiting known actions of known value and exploring new actions of unknown value, which can be higher or lower than the value of the known actions (Cohen et al., 2007; Sutton and Barto, 1998). In this context, the predictability of the environment might also influence the levels of behaviour variability

presented by an individual. If the environment is somehow constant and the sets of behavioural strategies with the highest values remain the same, it is likely that once an individual reaches one of these strategies after some action exploration – during which the individual should have high behaviour variability –, it will also likely exploit those strategies further and therefore reduce its behaviour variability. Conversely, if the environment is less predictable and the sets of behavioural values with highest value is changing often, it is likely that individuals will retain a high behaviour variability in the long run because even when they do find a high-value behavioural strategy in a given environment, that same behavioural strategy might later have a lower value when the environment changes and might no longer be exploited. As a consequence, the individual might then engage in action exploration once again.

Generation of behaviour variability has also been hypothesized to be a fundamental in the process of learning novel actions (Costa, 2011; Wu et al., 2014). More specifically, learning rate can be increased when initial behaviour variability is higher in the behaviour aspects relevant for the execution of a given task (Wu et al. 2014). This argument assumes that the ability to learn novel actions is also adaptive in itself, which can be the case, as documented by evidence gathered from experimental evolution (Mery and Kawecki, 2002). Thus said, if behaviour variability has an adaptive value, individuals with different levels of behaviour variability should have different fitnesses – *i.e.* behaviour variability should be under natural selection – and populations should be able to respond to that selection and behaviour variability should then evolve.

Detecting natural selection on phenotypes

The detection of natural selection on any set of phenotypes comes from the very simple premise that in a trait under selection, individual fitness is a function of the individual trait value. Therefore, a quantitative descriptor of selection such as the selection differential of a trait has been defined as a covariance between the trait and fitness (Price, 1970, 1972; Robertson, 1966). However, selection differentials only refer to the total amount of selection exerted on the trait and do not distinguish between direct selection on the trait and indirect selection due to correlation to another directly selected trait. Karl Pearson had actually set the ground to solve this issue by regressing traits on each other (Pearson, 1903), but a systematic approach was still needed. Russell Lande had actually started a general algebraic approach to measure direct natural selection on several traits. He defined selection coefficients as partial derivatives of fitness on the population trait means by modelling fitness as a multivariate function of the traits with an approximation obtained using a Taylor series (Lande, 1979). This approach was extended to describe individual relative fitness as a function of the individual multiple traits, in what was called the quadratic approximation of the individual selection surface (Lande and Arnold, 1983),

$$w = 1 + \sum_{i=1}^{n} b_i \, (z_i - \bar{z_i}) + \sum_{i=1}^{n} \sum_{j=1}^{n} \frac{1}{2} \gamma_{ij} (z_i - \bar{z}_i)(z_j - \bar{z}_j) \qquad (1.1)$$

This equation can be obtained through an ordinary least squares regression and describes the relative fitness of an individual, *w*, as a function of *n* traits, being $\bar{z_i}$ the population mean value of trait *i* and z_i the individual value of trait *i*. The intercept is fixed at the population mean

relative fitness of 1, the coefficient b_i is a linear coefficient of trait i and γ_{ij} are quadratic coefficients of the combination of traits i and j. When traits in combination follow a multivariate normal distribution, the linear and the quadratic coefficients are uncorrelated; in this case, the coefficients b_i are the selection gradients β_i, which are the linear coefficients of a linear ordinary least squares regression. Otherwise, selection gradients have to be obtained from a linear regression including only the linear terms (Walsh and Lynch, 2014),

$$w = 1 + \sum_{i=1}^{n} \beta_i \, (z_i - \overline{z_i}) \, . \qquad (1.2)$$

Selection gradients measure selection on the population phenotypic means. Their values are the slopes of the selection surface and quantify directional selection. If they are positive, selection favours an increase in the population mean phenotypic value (Figure 1.3a), whereas if they are negative, fitness declines according to population phenotypic mean and therefore selection favours a decrease in population phenotypic mean.

Quadratic selection measures selection on the population phenotypic covariances (Figure 1.3b,c,d). If $i=j$ in Equation 1.5, the coefficient γ_{ij} turns out to be a quadratic selection coefficient on trait i, which measures selection on the population phenotypic variance of trait i (Lande and Arnold, 1983; Phillips and Arnold, 1989). If the coefficient is negative, the fitness function is concave (Figure 1.3b,c,d), with a hilltop that is the fitness optimum and selection is considered stabilizing if the fitness optimum is inside the population fitness range (Figure 1.3b,d); if the quadratic selection gradient is positive, the fitness curvature is convex

and selection is disruptive if the fitness optimum is inside the population fitness range.

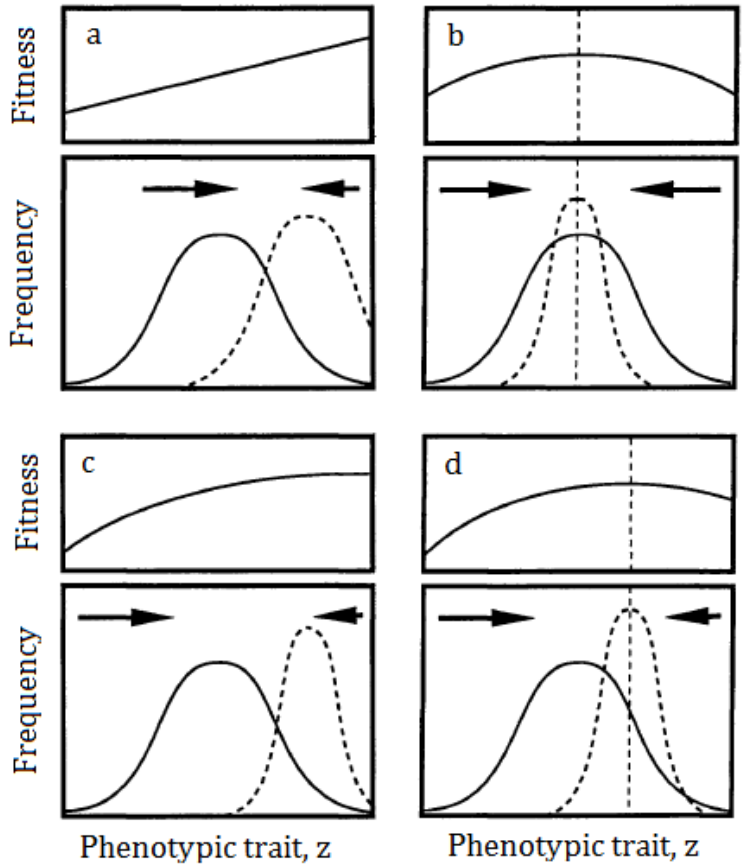


Figure 1.3 – Different modes of selection acting on a single trait. Solid, bell-shaped lines represent the trait distribution before selection; dashed bell-shaped lines are the trait distributions after selection, based on the fitness functions lying above. A straight dashed line indicates the fitness optimum when it is within the population phenotypic range. **a.** Directional selection, where the fitness function is linear and, therefore, the quadratic selection coefficient in Equation 1.5 would be zero. In this case, the selection gradient is positive, as fitness increases as the phenotypic value z increases. **b.** Stabilizing selection, where there is no linear selection. Therefore, only the population variance decreases, whereas the population mean does not change. In this case, the

quadratic selection coefficient is negative, as the fitness function is concave, and selection gradient would be zero. **c.** A combination of directional and non-linear selection. The selection gradient is positive and the quadratic selection coefficient is negative, but the fitness optimum is outside the population phenotypic range. **d.** A combination of directional and stabilizing selection. The coefficients can be similar to those in **c**, yet the fitness optimum is within the population phenotypic range. Both in **c** and **d**, selection acts both on population mean and variance. Adapted from Phillips and Arnold, 1989.

If $i \neq j$ in Equation 1.5, γ_{ij} will then be a correlational selection coefficient, which indicates selection on the population covariance between traits *i* and *j* (Figure 1.4). A positive coefficient can be interpreted as selection for increase in covariance between traits, whereas a negative coefficient can be interpreted as selection for decrease in covariance between traits.

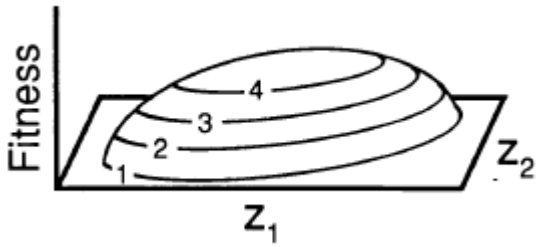


Figure 1.4 – Individual selection surface on two traits, z_1 and z_2, showing positive correlational selection between these two traits. Geometrically, correlational selection can be recognized when the major axis of the surface is not parallel to either of the phenotypic axes in question, which is the case here. Adapted from (Phillips and Arnold, 1989).

The simplicity of the quadratic approximation made its use very widespread in order to estimate selection coefficients and infer modes of

selection (see Kingsolver et al., 2001). However, selection coefficients thus obtained will be biased if there are environmental effects affecting the individual phenotypes and these covary with fitness. In such a case, the phenotypic differences that translate into fitness differences are not only rooted in the genetic effects on that phenotype but also in its environmental effects. To exclude the environmental effect on fitness, Mark Rausher has devised a modification of the quadratic approximation of the selection surface that allows estimation of selection coefficients that are based not on individual phenotypes, but on mean phenotypes of families of individuals, taking their genetic relationship into account (Rausher, 1992). These approximations of the selection surface are estimated by regressions that resemble the ones applied by Lande and Arnold for phenotypic selection (Lande and Arnold, 1983) but they look at selection on families instead of individual phenotypes. Therefore, the equations are very similar to equations 1.1 and 1.2, respectively, but with Rausher's notation they will take the forms below,

$$w = 1 + \sum_{i=1}^{n} B_i\,(\tilde{z}_i - \bar{\tilde{z}}_i) + \sum_{i=1}^{n}\sum_{j=1}^{n} \frac{1}{2} \Gamma_{ij} (\tilde{z}_i - \bar{\tilde{z}}_i)(\tilde{z}_j - \bar{\tilde{z}}_j) \qquad (1.3)$$

$$w = 1 + \sum_{i=1}^{n} B_i\,(\tilde{z}_i - \bar{\tilde{z}}_i)\,. \qquad (1.4)$$

In the equations above, directional selection pressures B and quadratic selection pressures Γ are, respectively, the linear and quadratic selection coefficients that result from regression of family-mean fitnesses on the family mean phenotypic values (Rausher, 1992). The regression coefficients B and Γ here estimated equal the coefficients β and γ estimated on phenotypes in the absence of environmental covariances

with fitness. The tilde emphasizes that these are family-mean values, not individual phenotypes.

Evolution of quantitative phenotypes

For any trait to evolve by selection – being natural or artificial selection –, selection acting on a population at a given generation has to lead to heritable phenotypic change in the following generation. If individuals with different phenotypes show different corresponding genotypes, then the trait has a genetic variance underlying it and this variance – measured along all individuals of a population – is useful to measure the degree of inheritance of a phenotype in an estimate we call heritability. The fraction of phenotypic variance (V_P) that corresponds to genetic variance (V_G) is the broad-sense heritability (H^2; (Falconer and Mackay, 1995),

$$H^2 = \frac{V_G}{V_P} \tag{1.5}$$

We can then partition genetic variance into three main variance components: the additive genetic variance (V_A), the dominance genetic variance (V_D) and the interaction or epistatic genetic variance (V_I). The additive variance is the only of these components which is heritable from parents to offspring and it is, thus, fundamental for any evolution to occur. The additive effects are the genetic effects expected to persist through segregation of parental genomes and recombination that gives rise to the offspring genomes. Based on the additive variance, we can also calculate a more strict heritability, the narrow-sense heritability (h^2),

$$h^2 = \frac{V_A}{V_P} \tag{1.6}$$

This narrow-sense heritability will be crucial in determining to what extent in a population the offspring will respond to selection taking place on their parents.

Heritability is a population property and therefore contingent on the population in question. This is because inheritance of a given phenotype from a given parent to its offspring depends on the other parent – drawn from that population – with which it is mating. In other words, the additive effects on a phenotype passed from one parent to its offspring – the breeding values (Falconer and Mackay, 1995) – depend on the other parent, which is sampled from the population, which has a given distribution of allelic frequencies. On the other hand, heritability of a phenotype will also depend on the environment, not only because the organism's surrounding environment affects the phenotype in a specific manner (Schlichting and Pigliucci, 1998), but also because the genetic effects on the phenotype depend on the environment (Falconer and Mackay, 1995; Yang, 2014).

Therefore, the response of a population to one generation of selection – *i.e.*, the change in population phenotypic mean – is contingent on the narrow-sense heritability and can be measured using the breeder's equation (Fisher, 1930), stated as

$$R = h^2 S \tag{1.7}$$

In this equation, S is the selection differential, which in context of natural selection is the covariance of the trait with fitness (Price, 1970, 1972; Robertson, 1966). However, traits should not be just looked in isolation, because natural selection occurs on individuals, which have not one, but an infinite amount of traits – including behaviours – that may be correlated to some degree and selected together. Therefore, as a given behaviour may contribute directly to natural selection, other behaviours will also be selected even if they do not contribute directly to fitness differences, for they are expressed in the same individuals, hence they are correlated to the first one. This first behaviour we say it is under direct selection and the others under indirect or correlated selection (Pearson 1903; Lande and Arnold 1983). Therefore, the breeder's equation as described in the above equation for one trait is not satisfactory to describe evolution of multiple traits. A multivariate version of the breeder's equation (Lande, 1979) can be written as the following matrix equation $\mathrm{R} = \mathbf{G}\beta$, extended below

$$\begin{bmatrix} R_1 \\ \vdots \\ R_q \end{bmatrix} = \begin{bmatrix} G_{11} & \cdots & G_{1q} \\ \vdots & \ddots & \vdots \\ G_{1q} & \cdots & G_{qq} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \vdots \\ \beta_q \end{bmatrix}, \tag{1.8}$$

where R is now a response to selection vector in q traits; each value reports the response to selection of a single trait. G is the additive genetic covariance matrix, which is a symmetric matrix of additive genetic covariances between traits. Its diagonal has the additive genetic variances of each trait (G_{11} to G_{qq}). The off diagonals are the additive genetic covariances between traits (G_{12} to G_{1q}). In quantitative genetics literature, it has been also widely known as the G-matrix (Phillips and McGuigan, 2006). β is the vector of directional selection gradients, which

describe the strength of directional selection by assessing to what extent fitness changes linearly as a function of each trait, after taking into account the correlations this trait may have with all other traits (Lande, 1979; Lande and Arnold, 1983).

Aims of this thesis

In the first place, this thesis aims to assess whether there can be natural selection acting on behaviour variability.

The question is, for instance, whether locomoting at a specific velocity or with a specific curvature are the only outputs of behaviour that are relevant for individual fitness or, as an alternative, whether locomoting at variable velocities and curvatures can also be relevant for that individual fitness. In the first case, an animal would engage in a somewhat constant or fixed behavioural strategy and exploring different strategies would not be of much relevance. In the second case, the animal could also explore different strategies in a given period of time or engage in several strategies during that time, besides possibly exploiting a given behavioural strategy. The expectation here was that when a population is exposed to a novel environment, its individuals would no longer respond behaviourally in an optimized manner that could be driven by a hard-wired neural network, in which case selection on behavioural variability, if present, would favour its decrease. Instead, the need to adjust behaviourally to the novel environment would imply the acquisition of vital experiences that would unlikely be hard-wired from birth because these populations had not experienced these environmental challenges before. By imposing a novel environment on these populations, we would

be, under this reasoning, exerting a selection pressure that would favour individuals that have more ability to incorporate experiences through learning and, because learning rate is higher on individuals that generate more behaviour variability, we would generate a selection favouring increased behaviour variability.

To accomplish this aim, I have used experimental populations of *Caenorhabditis elegans* that have undergone experimental evolution in a changing environment for 50 generations. From the ancestral population, I have derived inbred lines and I have performed univariate quadratic approximations of selection surfaces using both inbred line fecundity – used as a fitness component – and inbred line behaviour variability of a single behavioural feature at a time. Then, I have standardized the selection coefficients obtained by the behaviour mean of all inbred lines derived from the ancestral population and thus obtained elasticities of selection. These elasticities of selection allowed the comparison of selection coefficients among different behavioural features.

A second aim of this thesis is to compare selection on behaviour variability with selection on specific behaviour values. On one hand, as referred above, selection on behaviour variability would imply selection for enlarging or shrinking the range of possible behaviours that an individual can generate. If selection favoured increased behaviour variability, then individuals that explore a larger range of alternative behavioural strategies would have higher fitness. Conversely, if selection favours decreased behaviour variability, then selection might favour a decreased exploration of possible behaviours by each individual; instead, it might favour the exploitation of fewer behavioural strategies. On the other hand, a more canonical selection on behaviour implies selection on specific behavioural strategies put into practice by a given individual. The question here is which selection acting on behaviour matters the most,

whether it is selection acting on behaviour variability or selection acting on specific behaviours. For this purpose, I have estimated also univariate elasticities of selection for specific behaviour values using the inbred lines of the ancestral population and compared these estimates with elasticities of selection for behaviour variability in terms of their absolute magnitude. The more positive or negative these elasticities of selection on a given feature, the stronger the selection on that feature; conversely, the closer these elasticities of selection are to zero, the weaker selection is. Thus, to compare the strength of selection of behaviour variability features with the strength of selection on specific behaviour features, I have compared their absolute values of the elasticities of selection.

A third aim of this thesis is to assess effectively the adaptive value of behaviour variability. For that purpose, it is necessary to distinguish between direct and indirect selection acting on behaviour and behaviour variability. The univariate selection coefficients only quantify the total amount of selection acting on each feature and do not distinguish between selection acting directly on a feature from selection acting indirectly on a feature due to correlation with a directly selected feature. Therefore, I have also performed multivariate linear approximations of the selection surface using inbred line fecundity and all linear terms of all features, including both the features related to behaviour in itself and the features related to behaviour variability.

Finally, this thesis aims to analyse whether there was evolution of both behaviour and behaviour variability and how that evolution is related to the detected natural selection. Namely, this thesis aims to address the extent to which evolution of behaviour and its variability follow the directions pointed out by the estimated selection coefficients. To assess whether there was evolution of behaviour and its variability, the respective behavioural features were compared in the ancestral and

evolved populations. This comparison was also done using inbred lines, as the respective behavioural features in ancestral condition – measured in all the inbred lines derived from the ancestral population – were compared with the features in the evolved condition – using all inbred lines derived from all the evolved populations. The relationship between natural selection and evolution on all features was assessed by comparing the signs of the evolutionary responses observed – both in the populations and in the inbred lines – with the signs of both univariate and multivariate selection coefficients estimated for each feature. If the evolutionary responses were of the same sign of the selection coefficients, then evolution would have occurred in the direction pointed by the respective selection surfaces.

References

Attwell, D., and Laughlin, S.B. (2001). An Energy Budget for Signaling in the Grey Matter of the Brain. J. Cereb. Blood Flow Metab. *21*, 1133–1145.

Blanchard, R.J., and Blanchard, D.C. (1971). Defensive reactions in the albino rat. Learn. Motiv. *2*, 351–362.

Bond, C., and Harris, A.K. (1988). Locomotion of sponges and its physical mechanism. J. Exp. Zool. *246*, 271–284.

Chase, D., and Koelle, M.R. (2007). Biogenic amine neurotransmitters in *C. elegans*. WormBook.

Cohen, J.D., McClure, S.M., and Yu, A.J. (2007). Should I stay or should I go? How the human brain manages the trade-off between exploitation and exploration. Philos. Trans. R. Soc. Lond. B Biol. Sci. *362*, 933–942.

Costa, R.M. (2011). A selectionist account of de novo action learning. Curr. Opin. Neurobiol. *21*, 579–586.

Du, F., Zhu, X.-H., Zhang, Y., Friedman, M., Zhang, N., Uğurbil, K., and Chen, W. (2008). Tightly coupled brain activity and cerebral ATP metabolic rate. Proc. Natl. Acad. Sci. *105*, 6409–6414.

Elowitz, M.B., Levine, A.J., Siggia, E.D., and Swain, P.S. (2002). Stochastic Gene Expression in a Single Cell. Science *297*, 1183–1186.

Falconer, D.S., and Mackay, T.F.C. (1995). Introduction to Quantitative Genetics (Essex, England: Longman).

Fisher, R.A. (1930). The genetical theory of natural selection (Oxford University Press).

Fiske, D.W., and Rice, L. (1955). Intra-individual response variability. Psychol. Bull. *52*, 217–250.

Galea, J.M., Ruge, D., Buijink, A., Bestmann, S., and Rothwell, J.C. (2013). Punishment-Induced Behavioral and Neurophysiological Variability Reveals Dopamine-Dependent Selection of Kinematic Movement Parameters. J. Neurosci. *33*, 3981–3988.

Glaser, O. (1924). Temperature and forward movement of *Paramecium*. J. Gen. Physiol. *7*, 177–188.

Gomez-Marin, A., Paton, J.J., Kampff, A.R., Costa, R.M., and Mainen, Z.F. (2014). Big behavioral data: psychology, ethology and the foundations of neuroscience. Nat. Neurosci. *17*, 1455–1462.

Gordus, A., Pokala, N., Levy, S., Flavell, S.W., and Bargmann, C.I. (2015). Feedback from Network States Generates Variability in a Probabilistic Olfactory Circuit. Cell.

Hickman Jr., C.P., Roberts, L.S., and Keen, S.L. (2011). Animal Diversity (London: McGraw-Hill Higher Education).

Higham, T.E., Russell, A.P., and Zani, P.A. (2013). Integrative Biology of Tail Autotomy in Lizards. Physiol. Biochem. Zool. *86*, 603–610.

Holmes, S.J. (1903). Phototaxis in *Volvox*. Biol. Bull. *4*, 319–326.

Jin, X., and Costa, R.M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature *466*, 457–462.

Kandel, E., Schwartz, J., Jessell, T., Siegelbaum, S., and Hudspeth, A.J. (2012). Principles of Neural Science, Fifth Edition (McGraw Hill Professional).

Kao, M.H., Doupe, A.J., and Brainard, M.S. (2005). Contributions of an avian basal ganglia–forebrain circuit to real-time modulation of song. Nature *433*, 638–643.

Kety, S.S. (1957). The general metabolism of the brain *in vivo*. In Metabolism of the Nervous System, (London: Pergamon), pp. 221–237.

Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E., Hoang, A., Gibert, P., and Beerli, P. (2001). The Strength of Phenotypic Selection in Natural Populations. Am. Nat. *157*, 245–261.

Lande, R. (1979). Quantitative Genetic Analysis of Multivariate Evolution, Applied to Brain: Body Size Allometry. Evolution *33*, 402–416.

Lande, R., and Arnold, S.J. (1983). The Measurement of Selection on Correlated Characters. Evolution *37*, 1210.

Levitis, D.A., Lidicker Jr., W.Z., and Freund, G. (2009). Behavioural biologists do not agree on what constitutes behaviour. Anim. Behav. *78*, 103–110.

Mery, F., and Kawecki, T.J. (2002). Experimental evolution of learning ability in fruit flies. Proc. Natl. Acad. Sci. *99*, 14274–14279.

Messenger, J.B. (2001). Cephalopod chromatophores: neurobiology and natural history. Biol. Rev. Camb. Philos. Soc. *76*, 473–528.

Mosebach-Pukowski, E. (1937). Über die raupengesellschaften von Vanessa io und Vanessa urticae. Z. Für Morphol. Ökol. Tiere *33*, 358–380.

Nickel, M. (2004). Kinetics and rhythm of body contractions in the sponge Tethya wilhelma (Porifera: Demospongiae). J. Exp. Biol. *207*, 4515–4524.

Ohnishi, S. (1979). Relationship between larval feeding behavior and viability in *Drosophila melanogaster* and *D. simulans*. Behav. Genet. *9*, 129–134.

Ölveczky, B.P., Andalman, A.S., and Fee, M.S. (2005). Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol *3*, e153.

Omura, D.T., Clark, D.A., Samuel, A.D.T., and Horvitz, H.R. (2012). Dopamine Signaling Is Essential for Precise Rates of Locomotion by *C. elegans*. PLoS ONE *7*, e38649.

Pearson, K. (1903). Mathematical Contributions to the Theory of Evolution. XI. On the Influence of Natural Selection on the Variability and Correlation of Organs. Philos. Trans. R. Soc. Lond. Ser. Contain. Pap. Math. Phys. Character *200*, 1–66.

Phillips, P.C., and Arnold, S.J. (1989). Visualizing Multivariate Selection. Evolution *43*, 1209–1222.

Phillips, P.C., and McGuigan, K. (2006). Evolution of genetic variance-covariance structure. In Evolutionary Genetics: Concepts and Case Studies, C.C. Fox, and J.B. Wolf, eds. (Oxford University Press).

Price, G.R. (1970). Selection and covariance. Nature *227*, 520–521.

Price, G.R. (1972). Extension of covariance selection mathematics. Ann. Hum. Genet. *35*, 485–490.

Rausher, M.D. (1992). The Measurement of Selection on Quantitative Traits: Biases Due to Environmental Covariances between Traits and Fitness. Evolution *46*, 616–626.

Renart, A., and Machens, C.K. (2014). Variability in neural activity and behavior. Curr. Opin. Neurobiol. *25*, 211–220.

Robertson, A. (1966). A mathematical model of the culling process in dairy cattle. Anim. Sci. *8*, 95–108.

Santos, F.J., Oliveira, R.F., Jin, X., and Costa, R.M. (2015). Corticostriatal dynamics encode the refinement of specific behavioral variability during skill learning. eLife *4*, e09423.

Schlichting, C.D., and Pigliucci, M. (1998). Phenotypic Evolution: A Reaction Norm Perspective (Sunderland, Mass: Sinauer Associates).

Simmons, P., and Young, D. (2010). Nerve Cells and Animal Behaviour (Cambridge University Press).

Sokoloff, L. (1960). The metabolism of the central nervous system in vivo. In Handbook of Physiology, Section I, Neurophysiology, (Washington D.C.: American Physiological Society), pp. 1843–1864.

Sutton, R.S., and Barto, A.G. (1998). Reinforcement Learning: An Introduction (MIT Press).

Takikawa, Y., Kawagoe, R., Itoh, H., Nakahara, H., and Hikosaka, O. (2002). Modulation of saccadic eye movements by predicted reward outcome. Exp. Brain Res. *142*, 284–291.

Tinbergen, N. (1951). The study of instinct (Clarendon Press).

Tinbergen, N. (1963). On aims and methods of Ethology. Z. Für Tierpsychol. *20*, 410–433.

Tumer, E.C., and Brainard, M.S. (2007). Performance variability enables adaptive plasticity of "crystallized" adult birdsong. Nature *450*, 1240–1244.

Wadhams, G.H., and Armitage, J.P. (2004). Making sense of it all: bacterial chemotaxis. Nat. Rev. Mol. Cell Biol. *5*, 1024–1037.

Wagner, G.P., and Altenberg, L. (1996). Perspective: Complex Adaptations and the Evolution of Evolvability. Evolution *50*, 967–976.

Walsh, B., and Lynch, M. (2014). Genetics and Analysis of Quantitative Traits, Volume 2: Evolution and Selection of Quantitative Traits.

Weintraub, M. (1952). Leaf Movements in *Mimosa pudica* L. New Phytol. *50*, 357–382.

Wu, H.G., Miyamoto, Y.R., Castro, L.N.G., Ölveczky, B.P., and Smith, M.A. (2014). Temporal structure of motor variability is dynamically regulated and predicts motor learning ability. Nat. Neurosci. *17*, 312–321.

Yang, R.-C. (2014). Analysis of linear and non-linear genotype × environment interaction. Evol. Popul. Genet. *5*, 227.

Chapter 2: Materials and methods

I have used the nematode *Caenorhabditis elegans* as a model system to assess selection and evolution of behaviour and its variability. On one hand, this nematode has a very short generation time, allowing experiments involving many generations of evolution in a relatively short time span (Gray and Cutter, 2014). On the other hand, it is an outstanding model organism for the study of behaviour, because its brain anatomy is described with detail and behaviour measurement techniques have a very high resolution. Specifically, *C. elegans* has a very simple and anatomically described nervous system (Jarrell et al., 2012; White et al., 1986); the hermaphrodite neural connectivity, which involves 302 neurons, has been fully unveiled (White et al., 1986). Also, behaviour in *C. elegans* has been extensively and very accurately using very sophisticated tracking systems that allow behaviour measurement both of single individuals (Cronin et al., 2005; Feng et al., 2004; Stephens et al., 2008; Tsibidis and Tavernarakis, 2007) and multiple individuals at the same time (Ramot et al., 2008; Swierczek et al., 2011; Yemini et al., 2011).

In order to assess how behaviour and its variability can evolve, I have used an experimental evolution approach, in which evolution is performed in the laboratory by maintenance of populations throughout several generations and compared locomotion and postural patterns in the ancestral population and in the evolved populations. I have considered behaviour variability as a proxy for action exploration (Galea et al., 2013; Ölveczky et al., 2005; Wu et al., 2014) and analysed its evolution in a changing environment. I have measured behaviour using the Multi-Worm Tracker (Swierczek et al., 2011), which can record several behaviour features in multiple nematodes simultaneously.

To quantify selection on behaviour and its variability, I have derived inbred lines from the ancestral population, I have tracked locomotion and postural patterns and used inbred line fecundity measurements to estimate selection coefficients using the quadratic approximation of the individual selection surface (Lande and Arnold, 1983) mentioned in the introduction. This analysis was done both at the univariate and at the multivariate level.

Taking advantage of experimental evolution

For the study of the evolution of behaviour, I have taken advantage of an experimental evolution previously performed (Theologidis et al., 2014). This evolution has started from an ancestral population with standing genetic variation that has undergone experimental evolution to a constant laboratory environment (Chelo and Teotónio, 2013; Teotonio et al., 2012). The presence of standing genetic variation means that this ancestral population holds enough genetic variation so that it can evolve with a negligible contribution of mutation (Barrett and Schluter, 2008). This happens because the number of alleles introduced by mutation is very low when compared with the number of alleles already present in the population (Hill, 1982).

This ancestral population has undergone experimental evolution along 50 generations in a laboratory environment composed of Petri dishes with Nematode Growth Medium agar (NGM-lite, US Biological) with differing concentrations of sodium chloride (NaCl). In generation 0, the nematodes were raised in regular NGM-lite, which has NaCl at a concentration of 25 mM. Every generation, this concentration increased by 8 mM until generation 35, in which NaCl concentration was 305 mM. From generations 36 to 50, the abiotic environment remained stable and

NaCl concentration was stabilized at 305 mM (Figure 2.1). These populations have adapted to the gradual NaCl increase as fertility and mean fitness of these populations had already increased by generation 35 (Theologidis et al., 2014).

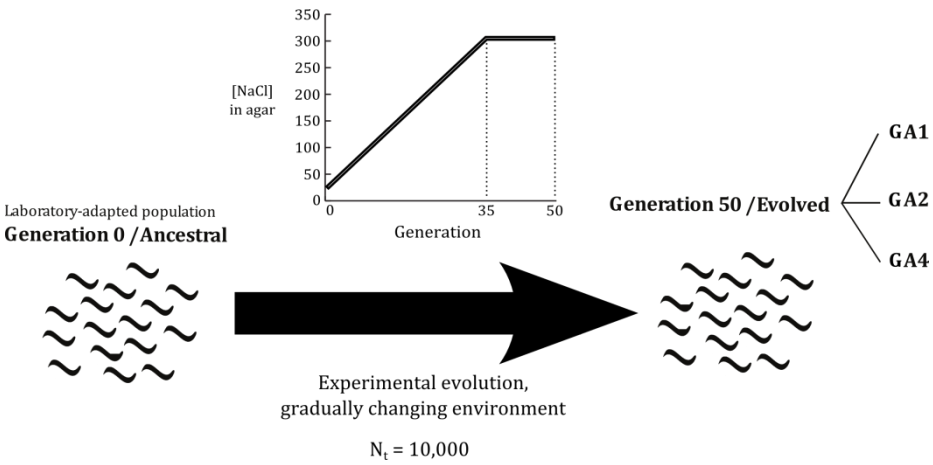


Figure 2.1 – The experimental evolution design as done in Theologidis et al., 2014. A population with standing genetic variation has undergone a 50-generation experimental evolution in a changing environment. In the first 35 generations, NaCl concentration in Petri dishes increased from 25 to 305 mM, at a constant rate of 8 mM per generation. From generations 36 to 50, NaCl concentration was kept stable at 305 mM. Three replicate populations have evolved, with constant size of 10,000 individuals every generation, distributed in ten Petri dishes of 1,000 individuals each. This number of individuals was estimated in the first larval stage (L1).

Sodium chloride has a well described physiological and behavioural effect on *C. elegans*. Sudden exposure of the N2 *C. elegans* strain to NaCl concentrations between 200 and 400 mM leads to body shrinkage due mainly to the osmotic pressure induced and the consequent water loss (Lamitina et al., 2004). The loss of turgor pressure inside the body of a

nematode leads also to a temporary locomotory defect, from which it recovers after some hours (Solomon et al., 2004). There is a response to the water loss in the nematode's body that involves the production of osmolytes, the main of which is glycerol (Lamitina et al., 2004). In our case, however, nematode exposure to NaCl is not sudden, but gradual along generations (Theologidis et al., 2014). Within any given generation from 1 to 35, nematodes were exposed to a single concentration of NaCl, which was only 8 mM higher than the concentration to which nematodes were exposed in the previous generation; likewise, in the following generation, NaCl concentration was only 8 mM higher. The effects of a gradual exposure to NaCl are less drastic than the effects of sudden transitions, because the nematodes can not only can acclimate to NaCl concentrations within a generation (Lamitina et al., 2004), but also along generations, because when parents are exposed to high NaCl concentrations they can increase survival of their offspring if these are also exposed to high NaCl concentrations or even higher (Frazier and Roth, 2009). In the context of the evolution of behaviour and its variability, two major points are worth pointing out. One is that this progressive increase allowed a constant environmental change that would eventually trigger the need to frequently explore novel actions that could help facing the environmental stress caused by the increasing osmotic pressure. The other is that a progressive NaCl concentration increase could mitigate selection pressures at the physiological level because *C. elegans* can physiologically acclimate to increasing NaCl concentrations if they are imposed gradually.

The population size was kept constant and estimated to be 10,000 individuals. Every generation was discrete and lasted four days. The first 24 hours were spent in 4 mL M9 liquid medium inside a 15 mL polypropylene tube, at 20 °C, under constant shaking at 120 rpm for aeration. During this period, embryonic development occured inside the

egg and the first larval stage hatches. Then, 10,000 individuals from each population are distributed by ten Petri dishes coated with *E.coli* HT115, each of which having 1,000 individuals transferred in the M9 solution. The nematodes will stay the following three days in these Petri dishes at 20 °C and 80% of relative humidity; during this time, the nematodes will resume development throughout the four larval stages until adulthood. In the end of this period, the nematodes are washed out with M9 solution and undergo a hypochloride treatment in a 1:1 ratio. This treatment will separate the current generation from the next, because it will sacrifice the adults, while allowing survival of the eggs that will form the following generation.

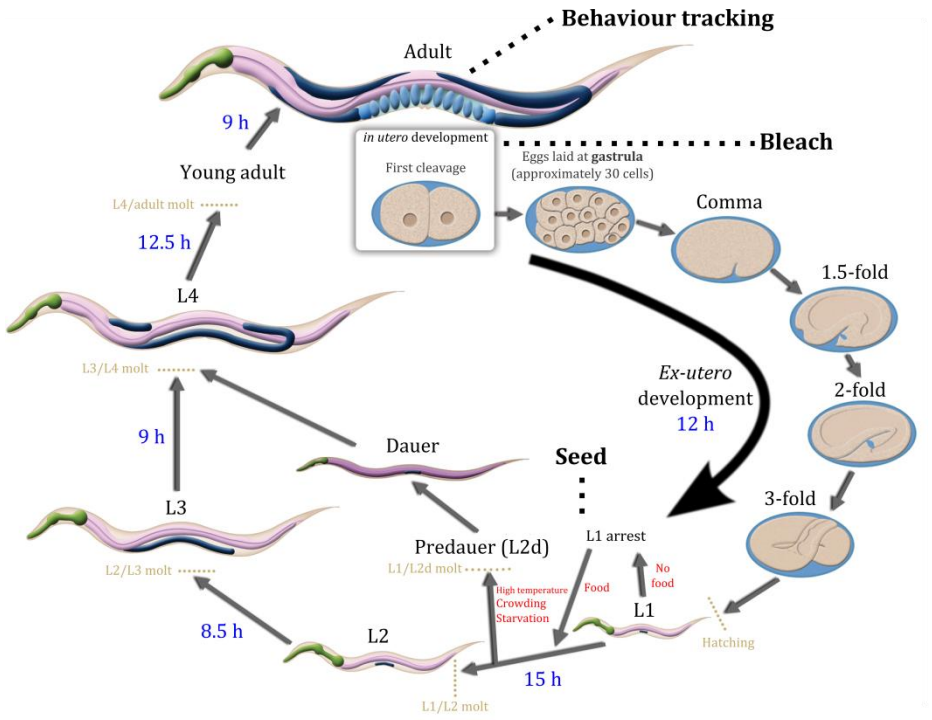


Figure 2.2 – The life-cycle of *Caenorhabditis elegans*. Each individual hatches from an egg as first-stage larva (L1) and goes through four larval stages – L1 to L4 – up to adulthood. Development will ensue regularly in the presence of food,

but it will arrest at L1 stage in the absence of food. If the environment is unfavourable for growth after L1 stage, the individual will develop under an alternative pathway into an arrested state termed as dauer, which follows a predauer stage (L2d). The dauer is a very thin larva with thick cuticle and can survive for months without food. Dauers do not appear in the experimental evolution at all; they only during population and inbred line handling in the context of behaviour tracking. The developmental times are based on measurements done at 20 °C, but without control on relative humidity (Byerly et al., 1976), thus they may be different from the ones that actually occurred in our experimental populations. The dashed lines remark the timing of seed, bleach and behaviour tracking, the last of which is explained in a section below. Adapted from (Altun and Hall, 2002).

Inbreeding ancestral and evolved populations from experimental evolution

Given the short life-cycle of *C. elegans*, it is experimentally unfeasible to measure on a large scale fecundity and behaviour in the same individual, in order to detect natural selection on behaviour and its variability. As an alternative, I have used behaviour and fecundity measurements of controlled genetic identities. Inbreeding is necessary to generate groups of individuals that share a single genetic identity – the inbred lines. Inbreeding was done by inducing self-fertilization in several consecutive generations. From each population, several hermaphrodites were isolated and placed in separate wells in 12-well plates, each of which had 3.5 mL of NGM agar (25 mM NaCl, with 100 mg/L ampicillin) and 2 µL spot of *E.coli* HT115. The *E.coli* spot was transferred from a liquid Luria Broth (LB) culture grown overnight from single-colony up to 10^7-10^8 cells. *E. coli* colonies were transferred from a LB agar Petri dish with 100 mg/L ampicillin to a 50 mL polypropylene tube with 10 mL LB with 100

mg/L ampicillin and 25 mg/L tetracyclin and incubated at 37 °C and 230 rpm for 16 hours. After adding the bacterial spot in the plates, these were incubated at 37 °C for 24 hours and stored at 4 °C for a maximum timespan of 2 weeks before usage.

Inbreeding resulted from random sampling of individual L4 hermaphrodites from each population to one well. The plates were then incubated at 20 °C and 80 % relative humidity throughout the whole generation time (4 days). After this time, the hermaphrodite has had several offspring at the fourth larval stage (L4) in each well as a result of self-fertilization, from which I sampled one to a new well to the following generation of inbreeding (Figure 2.3). If the hermaphrodite did not reproduce, another one was transferred from the respective well from the previous generation, which was stored at 4 °C. In this case, one generation of inbreeding was repeated. In absence of viable hermaphrodites at this previous generation, this lineage was considered as extinct and no further passage was done. This procedure was then repeated in every generation of inbreeding, until nearly complete homozygosity, *i.e.* a generation in which all individuals share the same genotypes and these are homozygote in every *loci*. After inbreeding, each inbred line was expanded up to at least 8,000 individuals, which were then frozen as L1 in liquid freezing solution (Stiernagle, 2006) and preserved at −80 °C in 1.5 mL cryopreservation tubes, each with 1 mL of solution with at least 2,000 L1 larvae.

Each individual sampled from the population can give rise to one inbred line by inbreeding in this fashion. This process is equivalent to maintaining populations of one individual, which means that in each generation the heterozygosity will theoretically decrease by half under neutrality (Crow and Kimura, 1970),

$$H_t = H_0\left(1 - \frac{1}{2N_e}\right)^t \qquad (2.9)$$

where H_t and H_0 are the heterozygosities at generations 0 and t, respectively, and N_e the effective population size. This equation states that heterozygosity of a population will decrease as a function of its effective size N_e. In population genetic terms, the inbreeding process was a decrease in population size to 1 individual (N_e = 1 in equation 2.1) and each of these populations will give rise to one inbred line. As the inbreeding proceeded for 12 generations, the effective population size of each inbred line was 1 during 12 generations.

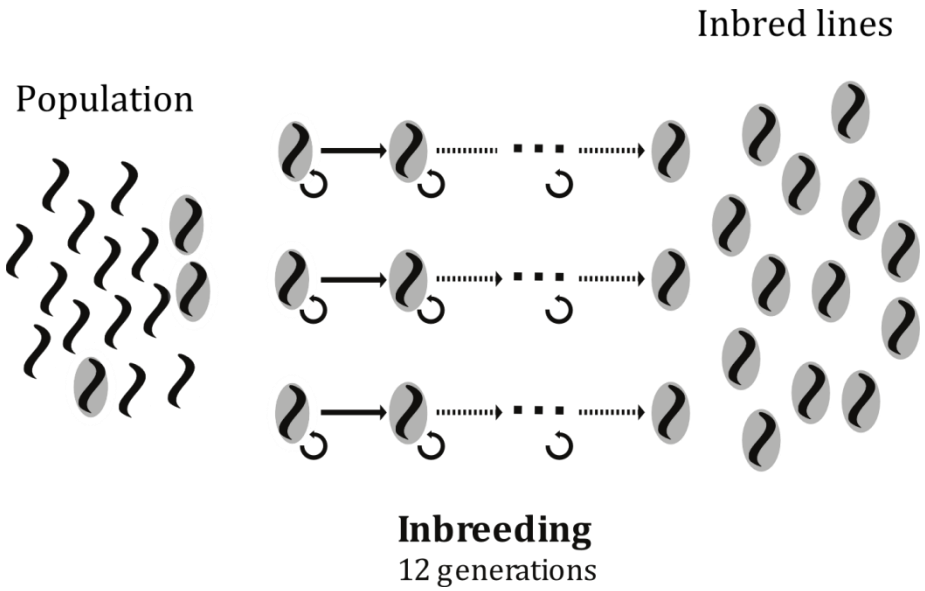


Figure 2.3 – The inbreeding scheme. From each population, several hermaphrodites were taken (individuals in the population inside grey ellipses), each of them to form one inbred line. Each of these hermaphrodites has gone through self-fertilization and, from their offspring, one hermaphrodite was taken to form the next generation. This process was repeated for at least 12

generations, after which I have considered to have obtained one inbred line from each hermaphrodite that came from the original population.

Since there is only asymptotic homozygosity with inbreeding, I wanted to check the level of heterozygosity left in the inbred lines. Also, because I wanted to compare inbred lines coming from populations in different generations of evolution, it was essential to make sure there was no difference in inbreeding extent among the inbred lines coming from different generations that could justify the evolutionary responses observed. Therefore, I have taken advantage of the 830 SNP genotyping that has been done by Ania Pino. In each inbred line, DNA was genotyped from a pool of individuals grouped together. For each inbred line, I have calculated heterozygosity as the fraction of genotyped SNPs that were heterozygote and found inbred lines with outlying levels of heterozygosity in the ancestral inbred lines (Figure 2.4). These lines with excessive heterozygosity might have resulted from admixture between different inbred lines during the process of inbreeding, especially during the population expansion of each inbred line prior to their storage by freezing.

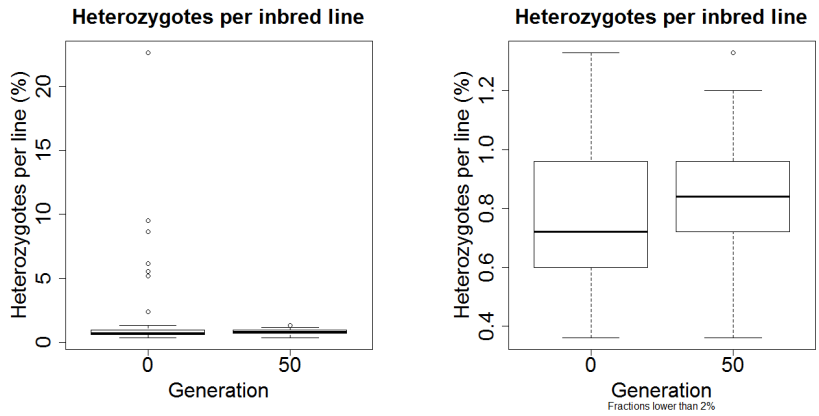


Figure 2.4 – Heterozygosity in ancestral inbred lines (generation 0) and evolved inbred lines (generation 50). Heterozygosity here is measured as the fraction of genotyped SNPs that were heterozygote in a given inbred line. Left, heterozygosities in all inbred lines, showing some inbred lines with very high heterozygosities, especially in the ancestrals. Right, when analysing only inbred lines with heterozygosities lower than 2%, heterozygosity distributions in the ancestral and in the evolved inbred lines (t = 1.311; d.f.: 312; P = 0.1909). The inbred lines under analysis in this plot were the ones kept for further analyses.

Given this scenario, I have introduced an acceptable heterozygosity threshold above which an inbred line should be discarded by excessive heterozygosity and that threshold was 2%. After excluding the inbred lines with heterozygosity higher than 2%, the levels of heterozygosity in these inbred lines are equivalent both when coming from ancestral and evolved populations (Figure 2.4). In the end of this process, I had 180 inbred lines derived from the ancestral population 140A6 and 131 inbred lines derived from the evolved populations – 50 from GA1, 51 from GA2 and 30 from GA4, all of them at generation 50 of experimental evolution; the evolved populations were thus named as they represent androdioecious (A) populations that have undergone evolution in a

gradually (G) changing environment (Theologidis et al., 2014). This set of inbred lines was the departure for all following analyses in this thesis.

Tracking behaviour using the Multi-Worm Tracker (MWT)

To record behaviour of the nematodes, I have used a computer vision approach to record their locomotory behaviour. I have replicated the Multi-Worm Tracker (MWT; Swierczek et al., 2011), which is a real-time tracking system that allows tracking of several nematodes simultaneously in a given Petri dish. As in the original work, the hardware used to make the MWT was a CCD camera Dalsa Falcon 4M30, a Schott backlight A08926 that allowed homogeneous illumination along the plate being tracked. The camera was connected to a 4 GB RAM computer through a National Instruments PCIe-1427 CameraLink capture card. However, no stimulus triggering equipment was installed, because the aim was to track freely moving nematodes. MWT has a LabView interface, which allows control of general tracking settings.

To minimize external light reflection, a 45x58 mm rectangle was cut in a 90-mm plate lid using a laser cutter (Epilog) and replaced by a 48x60 mm cover glass (Gold Seal) using UHU contact glue, and tracking was only performed after replacement of the respective plate lid by this previously prepared lid.

Tracking was performed for 25 minutes, with both contours and skeletonization enabled in the MWT. Enabling contours allowed me to save both the contours of each individual, i.e. the points that circumscribe each individual nematode, and enabling skeletonization allowed me to save eleven points along the spine, which is the mid-longitudinal line

computed along the length of the individual. These computations allowed me to measure behaviour that is based on shape changes in time.

An object under the MWT is defined both by its contrast with the background and by its fill hysteresis, both of which setting up a dual threshold that defines a more central part of the object (contrast) and a more peripheral one (fill hysteresis). Contrast here is defined as difference in intensity between neighbouring pixels. Since the tracking camera is monochromatic, there is only one scale of pixel intensity that visually corresponds to a greyscale and goes from black (minimum intensity) to white (maximum intensity). In this system, illumination comes from below, travels across both the plate and the agar before hitting the camera. As a consequence, the individual nematodes will appear as dark objects on a light background. Fill hysteresis defines the lower contrast level with the background and thus it defines how an object is formed, starting from the central region pixels, of higher contrast, towards the peripheral region, of lower contrast. Once MWT detects an object, it can also tolerate some variation in size due to camera noise and that variation can be set up in the 'object size hysteresis' parameter. This also allows a stable tracking of objects that are close to the established size limits, otherwise they would get in and out of tracking according to the noise, which would result in many short-time tracks.
MWT also allows us to establish minimum and maximum size thresholds in order to validate an object. These values represent the minimum and maximum amount of pixels an object can have.

All these parameters in MWT were kept default – namely minimum object size of 50 pixels, maximum object size of 6000 pixels, 10 % object contrast, 50 % fill hysteresis, 10 % object size hysteresis. I shall explain the contrast and hysteresis settings with a numerical example. Being this

a 10-bit camera, it has a dynamic range of 0 to 1023 in intensities; if, for instance, the background intensity is 812, the 10% object contrast means that the MWT will consider an object of intensity lower than the background in 10% of the dynamic range, i.e. in 102; therefore, it will consider objects with intensities lower than 710 (812 – 102). On the other hand, the 50% fill hysteresis means that the contour of the object will lie below the background in 50% of the contrast, i.e. in light intensity that is below background in 51 (0.5 × 102), which means that the contour will lie in intensity of 761 (812 – 51). Therefore, besides having a size of 50 to 6000 pixels, any valid object will be filled from a central part with light intensity below 710 outwards to a light intensity of 761 in its contours. Also, if this object has a size of, say, 242 pixels at the onset of tracking, it can vary in size in 20% means that an object can vary in size up to 20% higher or 20% lower than this size.

These settings seem to be the most adequate given what I have observed after tuning and looking at the resulting worms tracked on the MWT interface. The interval of object sizes allowed me to capture the worm size diversity I would likely have in different genotypes.

Experimental design for *C. elegans* tracking

Both populations and inbred lines were revived from freezing into a 27 mL NGM-lite 90 mm Petri plate coated with a lawn of HT115 *E.coli*. These plates were stored for a maximum of 4 weeks at 4 °C before usage. Nematodes were then maintained for two generations in the same Petri plate, at 20 °C and 80 % relative humidity, in order to control for the effects of freezing. After five days, the revived nematodes were washed out of the plate using an isotonic M9 solution. At this point, I had the adult

hermaphrodites from the revived generation as well and their offspring in many different larval stages. All worms were centrifuged twice at 8 rcf to drag adults to the tube bottom and facilitate their removal by pipetting out the resulting pellet from each centrifugation. The remaining L1/L2d/dauers (see Figure 2.2) were scored live in 5 samples of 5 µL each, to estimate the volume necessary to get 1,000 individuals, which were seeded in each of three NGM plates. These plates were incubated at 20 °C and 80 % of relative humidity. Three days later, the resulting adults were washed out of the plates with M9 solution and exposed to a 40 mM KOH (Sigma-Aldrich): 1.2% NaClO (Roth) solution ("bleach" solution), in a 1:1 volumetric ratio. This treatment lasts for 5 minutes and these include one centrifugation at 652 rcf during the last minute. The resulting pellet, which includes adult debris and eggs, was pipetted to a new tube with 10 mL of M9 for the first of three M9 washes. The remaining two washes were done by two cycles of 652 rcf, 1 minute centrifugation, M9 supernatant discard and refill up to 10 mL. After the third wash and discard, M9 solution was added to a volume of 4 mL and each tube was incubated at 20 °C, 80 % RH and 120 rpm shaking condition for the following 24 hours, during which the eggs hatch. After this time, tubes were centrifuged at 8 rcf and the adults were then pipetted out. The number of remaining L1 larvae was scored live using, again, 5 samples of 5 µL and seeding volume was estimated for 1,000 individuals. These were seeded for tracking both in NGM 25 mM and NGM 305 mM NaCl. In summary, two generations have passed in NGM between revival and tracking, therefore behaviour tracking was done on each environment in the third generation after revival.

All maintenance procedures were taken in two time periods, a morning one and an afternoon one. In the morning time, revivals were done at 1:30 p.m., washes and seedings after reviving started at 11 a.m., bleaches started at 1 p.m. and seedings for tracking were done at 2 p.m. In the

afternoon, the respective times for the same procedures were 7:30 p.m., 5 p.m., 7 p.m. and 8 p.m.. Inbred line plates were replicated twice, whereas the populations were replicated 10 times. Each tracking plate mimicked exactly the biotic conditions that occurred in experimental evolution, namely in terms of nematode and *E. coli* densities (Theologidis et al., 2014).

Tracking was performed in morning – starting at 8 a.m. – and afternoon blocks – starting at 2 p.m. –, starting two days and 18 hours after the aforementioned seeding, being the worms 3.75 days old. This timing was chosen in order to track adult worms at an age within their usual life expectancy during experimental evolution (4 days old). Inbred lines and populations were shuffled in sequence in each of the morning and afternoon blocks, but each inbred line / population was tracked in both environments consecutively, with randomized environment order within each. The tracking plates were seeded with ~1,000 individuals, the same number of individuals existing on each plate during experimental evolution.

Many were the factors I could not control in order during the experimental setup and these I have measured and used as covariates in the statistical models. As there is no feasible way of handpicking 1,000 individuals repeatedly for several plates and several inbred lines, I have counted five 5-µL samples of swimming L1 larvae in M9 solution using a light stereoscope and, from these counts, I have estimated the volume of M9 with larvae that would be necessary to seed each plate with 1,000 worms. However, this procedure is coupled with a non-negligible amount of error and all plates will have a different number of nematodes. These differences in nematode density could definitely influence both their behaviour and developmental rate (Yamada et al., 2010); for this reason, I have counted the number of individuals in each tracked plate using a light

stereoscope and a printed 90 mm circular grid. In the population plates, numbers of both hermaphrodites and males were counted.

In the area on the tracking plate where the volume of M9 with L1 larvae is transferred to, a very dense *E.coli* lawn usually forms. This phenomenon is common throughout experimental evolution as well and generates a heterogeneity in the environment, because there will be bacteria lawns with two markedly different densities. *C. elegans* has dietary preferences (Shtonda and Avery, 2006) and also behaves differently according to the density of the bacterial lawn it finds (Bendesky et al., 2011). Therefore, I have recorded the volume of M9 transferred in order to have 1,000 individuals on the tracking plate. This volume should be a proper indication of the extent of high density *E.coli* existing on a given tracking plate. Because *C. elegans* displays also strong maternal effects on behaviour, especially in what concerns to food intake (Tauffenberger and Parker, 2014; Yu et al., 2013), I have also recorded the M9 volume added in order to transfer 1,000 individuals to the plates of the parental generation, i.e. the generation of the parents of the individuals being tracked.

In my assays, I also had no control over temperature or relative humidity. Temperature has a broad influence over development and behaviour in *C. elegans*. For instance, *C. elegans* development is 50% faster at 25 °C than at 20 °C and *C. elegans* velocity is also higher at higher temperatures (Hope, 2000). Also, *C. elegans* locomotion is broadly affected by relative humidity, namely, reverse locomotion frequency is higher in more humid environments (Zhao et al., 2003). For these reasons, both temperature and relative humidity were measured immediately after tracking using a thermometer/relative humidity meter. All plates in each block were seeded at the same time, but they were tracked sequentially. Therefore, different inbred lines and populations have random differences in the

developmental stages in which they are tracked. I have recorded the order in which the inbred lines and populations were tracked in each block using integer numbers.

Quantification of the behavioural features

Tracking data was processed offline using the java-based analysis software Choreography, which was also developed in order to function with the MWT data (Swierczek et al., 2011). To run Choreography, I have used a 16 Gb RAM computer using the command written below,

java -jar -Xmx6G -Xms6G $HOME/Chore.jar -S --shadowless -q --plugin Reoutline --plugin Respine -o id,persistence,area,speed,angular,length,width,aspect,midline, morphwidth,bias,pathlen,curve,loc_x,loc_y -N all.

In other words, I used 6 Gb of RAM memory for Choreography analysis in each plate, with Reoutline and Respine plugins and extracted a worm ID – a specific integer that identifies the worm track – as well as individual worm measurements for track persistence, area, velocity (speed), turning rate (angular speed), regressed length (length), regressed width (width), aspect, worm length (midline), worm width (morphwidth), locomotion bias (bias), net distance travelled (pathlen), mean body curvature (curve), positions in *x* and *y* axes (loc_x and loc_y, respectively). The Reoutline plugin smoothens the outline of the nematodes and the respine plugin recomputes the nematode's eleven points of the spine, given its outline. The individual measurements extracted are defined in the Table 2.1, lying below.

Table 2.1 – Description of the behavioural features used directly from the Choreography output for analysis. Feature abbreviations used in plots are in parentheses.

Behaviour	**Description**	**Units**	**MWT designation**
Velocity (V)	Worm maximum centroid displacement per unit of time. It is calculated using 0.5 second bins in a sliding window of time. Specifically, v_t is the distance between the most distant worm positions the worm has been in between times *t* and *t-0.5* (see Figure 2.5).	mm/s	Speed
Turning rate (TR)	Change in worm orientation using 0.5 second bins, in a sliding window of time used in the same way as in velocity calculation. The worm orientation, is defined by an ordinary least squares regression line of the worm pixels and the change in orientation is measured by the largest angle, in degrees, defined between the regression lines calculated at all worm positions between times *t* and *t-0.5* (see Figure 2.6).	deg./s	Angular speed
Regressed length (RL)	Length of the line defined by the ordinary least-squares fit of the major, longitudinal axis of the worm pixels (Figure 2.7).	mm	Length
Regressed width (RW)	Length of the line defined by the ordinary least-squares fit of the minor, transversal axis of the worm pixels. This axis is perpendicular to the major axis, used for the regressed length (Figure	mm	Width

	2.7).		
Net distance travelled (NDT)	Cumulative distance travelled by the worm in forward locomotion, minus the distance travelled backwards.	mm	Path length
Curvature (C)	Mean angle between worm parts along its skeleton, which is divided into five equal segments. Thus said, this is the mean of four angles (Figure 2.8).	deg.	Curve
Aspect (A)	Ratio between regressed width and regressed length.		Aspect

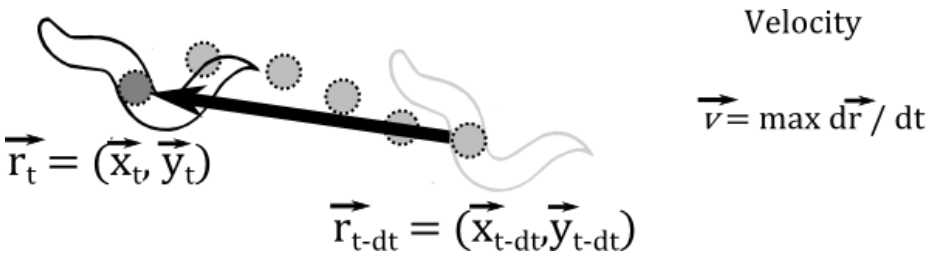


Figure 2.5 – Illustrative scheme of velocity measured in each nematode by the MWT and explained in Table 2.1, lying above.

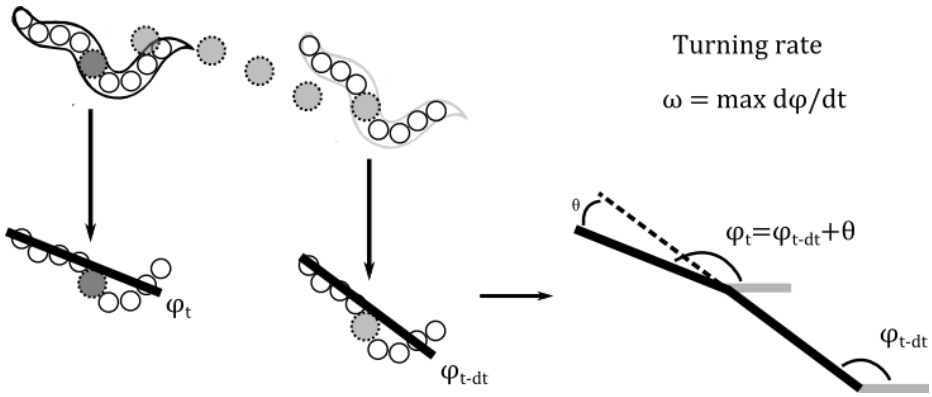


Figure 2.6 – Illustrative scheme of turning rate measured in each nematode by the MWT. The nematode orientation, defined as an ordinary least squares of the

are drawn in black after the arrows facing down and in this example $d\varphi$ is equivalent to θ. A more detailed description lies in Table 2.1 above.

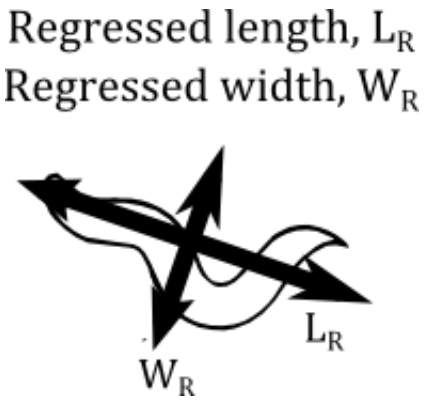


Figure 2.7 – Illustrative scheme of both regressed length (L_R) and regressed width (W_R), explained in detail in Table 2.1.

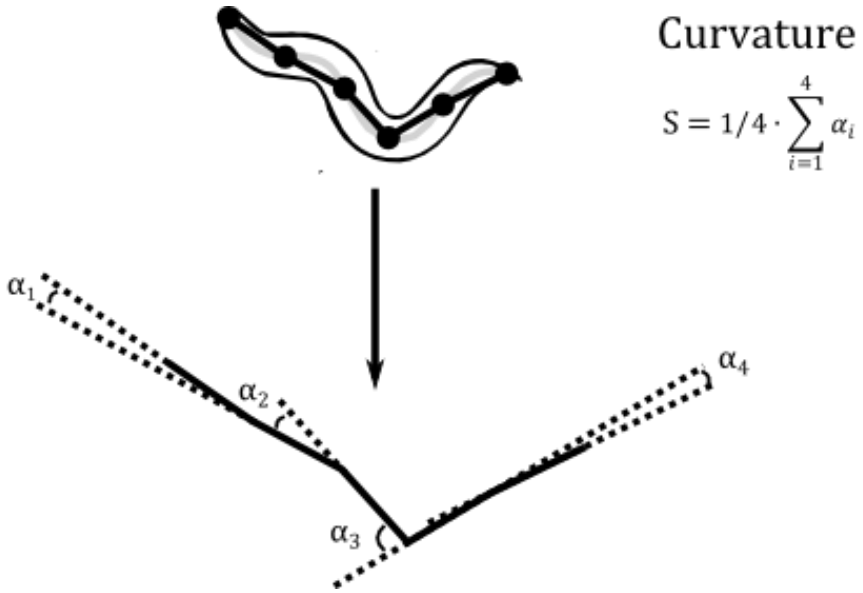


Figure 2.8 – Illustrative scheme of curvature, showing the four angles that are averaged. More details are in Table 2.1.

Also, I have taken some measurements other than behavioural on the worms from Choreography – Table 2, below – in order to generate other behavioural measurements in combination with features described in Table 2.1.

Table 2.2 – Other measurements extracted by Choreography, which were used to generate other behavioural features.

Measurement	**Description**	**MWT designation**
Persistence	Time during which the worm was tracked.	Persistence
Area	Number of pixels considered as being part of the worm body.	Area
Length	Length of the skeleton taken along the midline of the worm (schematized in Figure 2.9).	Midline
Width	Mean width of all segments perpendicular to the skeleton (black in Figure 2.9) in the central 60% of the worm body.	Morphwidth
Locomotion bias	States the direction of worm locomotion. At each frame, it can have the values of 1, 0 or -1, depending on whether the worm is in forward locomotion, stationary or in reverse locomotion, respectively. Head and tail not distinguished in the MWT, so the tracker assumes the dominant locomotion direction is forward. This is a quite fair assumption	Bias

	given previous quantifications of *C. elegans* locomotion (Shingai, 2000).	
X-position	Worm position in the x-axis.	X location
Y-position	Worm position in the y-axis.	Y location

Figure 2.9 – Scheme illustrating length and width measurements (thick black lines) made by the MWT on each individual nematode and described in detail on Table 2.2, immediately above.

All other measurements I have used in the analysis came from secondary calculations, taking the ones mentioned in Tables 1 and 2 as a starting point. Forward measurements are measurements taken when the worm was moving forward – i.e. when locomotion bias was 1 –, reverse measurements are the ones taken when the worm was reversing – i.e. when locomotion bias was -1 – and stationary measures are the ones corresponding to locomotion bias of 0, when the worm is considered to be immobile. The behavioural features generated from the Choreography extracted features, described in Table 2.1 and Table 2.2 – are described in Table 2.3.

Table 2.3 – Behavioural features generated from the measurements that came as an output of Choreography and described in Tables 2.1 and 2.2. All forward, reverse and stationary features refer to the periods in which the locomotion bias is 1, -1 or 0, respectively. Abbreviations were used to represent features in plots of this thesis.

Behaviour	**Abbreviations**	**Description**
Forward locomotion bout duration	FwdDuration, FDr	Time between each forward bout initiation and termination.
Reverse locomotion bout duration	RevDuration, RDr	Time between backward locomotion bout initiation and termination.
Interval between locomotion bouts	BoutInterval, BI	Time between termination of a bout and the initiation of the following bout, regardless of bout direction.
Interval between forward bouts	FwdInterval, FI	Time between termination of a forward bout and the initiation of the next forward bout.
Interval between reverse bouts	RevInterval, RI	Time between termination of a reverse bout and the initiation of the next reverse bout.
Overall locomotion fraction	LocomotionFraction, LFc	Fraction of tracking time spent in active locomotion, i.e., ratio between the sum of all bout durations and track persistence (see also Table 2.2).

Forward locomotion fraction	FwdFraction, FFc	Fraction of tracking time spent in forward locomotion, i.e., ratio between the sum of all forward bout durations and track persistence (see also Table 2.2).
Reverse locomotion fraction	RevFraction, RFc	Fraction of tracking time spent in reverse locomotion, i.e., ratio between the sum of all reverse bout durations and track persistence (see also Table 2.2).
Forward bout frequency	FwdFrequency, FFq	Number of forward bouts per unit of time. It is calculated as the number of forward bouts divided by track persistence[2] (see Table 2.2).
Reverse bout frequency	RevFrequency, RFq	Number of reverse bouts per unit of time. It is calculated as the number of reverse bouts divided by track persistence[2] (see Table 2.2).
Forward locomotion velocity	FwdVelocity, FV	Worm velocity (Table 2.1) when in forward locomotion.
Reverse	RevVelocity, RV	Worm velocity (Table 2.1)

[2] Bout frequencies were measured as number of bouts per minute. Hence, persistences were converted to minutes by dividing the original track persistence values (in seconds) by 60.

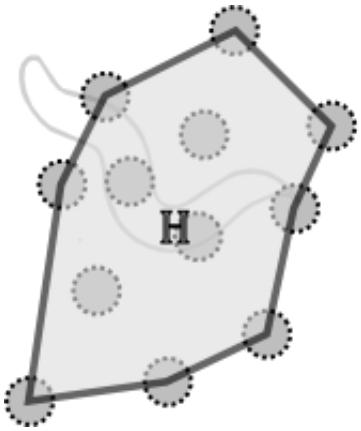
locomotion velocity		when in reverse locomotion.
Turning rate during forward locomotion	FwdTurningRate, FTR	Worm turning rate (Table 2.1) when in forward locomotion.
Turning rate during reverse locomotion	RevTurningRate, RTR	Worm turning rate (Table 2.1) when in reverse locomotion.
Stationary turning rate	StatTurningRate, STR	Worm turning rate (Table 2.1) when not in locomotion.
Curvature during forward locomotion	FwdCurvature, FC	Worm curvature (Table 2.1) during forward locomotion.
Curvature during reverse locomotion	RevCurvature, RC	Worm curvature (Table 2.1) during reverse locomotion.
Stationary curvature	StatCurvature, SC	Worm curvature (Table 2.1) when not in locomotion.
Aspect during forward locomotion	FwdAspect, FA	Worm aspect (Table 2.1) during forward locomotion.
Aspect during reverse locomotion	RevAspect, RA	Worm aspect (Table 2.1) during reverse locomotion.
Stationary aspect	StatAspect, SA	Worm aspect (Table 2.1) when not in locomotion.
Regressed length during forward	FwdRegLength, FRL	Regressed length (Table 2.1) when the worm is in forward locomotion.

locomotion		
Regressed length during backward locomotion	RevRegLength, RRL	Regressed length (Table 2.1) when the worm is in reverse locomotion.
Stationary regressed length	StatRegLength, SRL	Regressed length (Table 2.1) when the worm is not in locomotion.
Regressed width during forward locomotion	FwdRegWidth, FRW	Regressed width (Table 2.1) when the worm is in forward locomotion.
Regressed width during reverse locomotion	RevRegWidth, RRW	Regressed width (Table 2.1) when the worm is in reverse locomotion.
Stationary regressed width	StatRegWidth, SRW	Regressed width (Table 2.1) when the worm is not in locomotion.
Longitudinal bending during forward locomotion	FwdLongitudinalBending, FwdLongBending, FLgB	The ratio between worm length (Table 2.2) and worm regressed length (Table 2.1) when the worm is in forward locomotion.
Longitudinal bending during backward locomotion	RevLongitudinalBending, RevLongBending, RLgB	The ratio between worm length (Table 2.2) and worm regressed length (Table 2.1) when the worm is in reverse locomotion.
Stationary longitudinal	StatLongitudinalBending, StatLongBending, SLgB	The ratio between worm length (Table 2.2) and

bending		worm regressed length (Table 2.1) when the worm is not in locomotion.
Lateral bending during forward locomotion	FwdLateralBending, FwdLatBending, FLtB	The ratio between worm regressed width (Table 2.1) and worm width (Table 2.2) when the worm is in forward locomotion.
Lateral bending during reverse locomotion	RevLateralBending, RevLatBending, RLtB	The ratio between worm regressed width (Table 2.1) and worm width (Table 2.2) when the worm is in reverse locomotion.
Stationary lateral bending	StatLateralBending, StatLatBending, SLtB	The ratio between worm regressed width (Table 2.1) and worm width (Table 2.2) when the worm is not in locomotion.
Distance travelled in reverse locomotion	RevDistance, RDt	The sum of position changes during reverse locomotion.
Distance travelled in forward locomotion	FwdDistance, FDt	The sum of net distance travelled (Table 2.1) and distance travelled in reverse locomotion.
Total distance travelled	TotalDistanceTravelled, TDT	The net distance travelled plus twice the distance travelled during reverse

		locomotion.
Exploration rate	ER	The area of the region described by the nematode centroid positions per unit of time. This area was calculated as the area of the shortest convex region that includes all tracked x- and y-positions of the nematode, *i.e.* the area of the convex hull of x- and y- positions of the nematode. This calculation was accomplished using R functions 'chull' and 'areapl', the latest from 'splancs' R package (Rowlingson and Diggle, 2013). This area of region explored divided by the nematode track persistence, in minutes (see also Table 2.2; a scheme is in Figure 2.10).
Exploration density	ED	The area of the nematode trace, defined as a thick line that includes its trajectory, divided by the exploration rate. Nematode trace area was

		obtained by $D_{total} \times w$, the product of the total distance travelled and the worm width (see also Table 2.2).
Distance to average nearest neighbour	DistanceNearestAvgNeighbour, DNN	The distance between the average position of the worm during the whole tracking time and the average position of the nearest neighbouring worm (Figure 2.11).
Distance to average nearest neighbour, standardized	DistanceNearestAvgNeighbourStd, DNNS	Distance to average nearest neighbour standardized by the number of worms tracked on plate.



Exploration rate

$$E = A_H / dt$$

Figure 2.10 – Illustration of exploration rate. The convex hull (H) is emphasized in light grey and delimited by the points lining in the dark grey line. More details on this measurement are in Table 2.3 above.

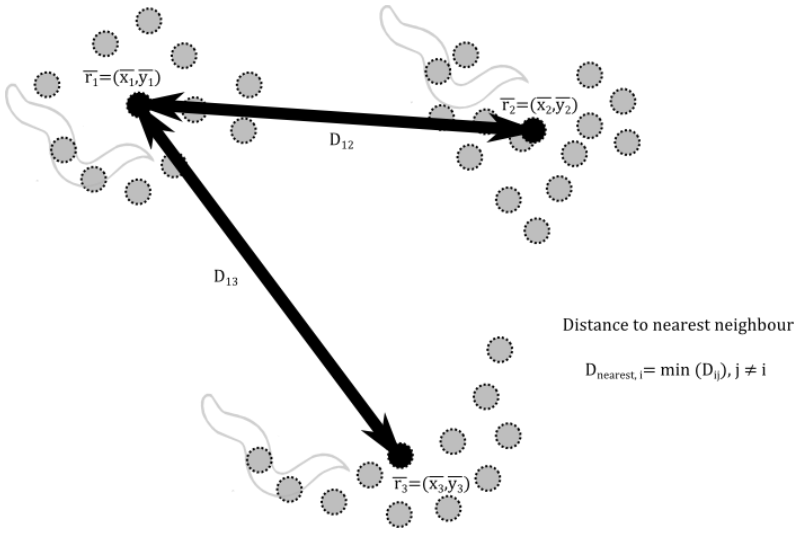


Figure 2.11 – Illustration of distance to nearest neighbour. In this example, three individual nematodes are depicted and all their positions in a given interval of time as pointed in grey. The mean position of each nematode throughout the observation time is shown in black and named as $\overline{\boldsymbol{r}_{\boldsymbol{i}}}$. The smallest of these distances, marked in black arrows, is the distance to nearest neighbour. More details are in Table 2.3 above.

To get the number of worms tracked at each frame, I have used the command *java -jar -Xmx6G -Xms6G $HOME/Chore.jar -S --shadowless -q --plugin Reoutline --plugin Respine -o N*.

Behaviour statistics under analysis

I have used two elementary summary statistics of each behavioural feature in each individual. To look for behaviour evolution in general, I have used the median of the measurements taken along the worm track

during the sampling time. This facet of behaviour will also be termed as behaviour centrality along this thesis. On the other hand, to look at behaviour variability, I have used the median absolute deviation from the median (MAD) to quantify behaviour variability within a worm track along time,

$$MAD = |\widetilde{x - \tilde{x}}| \tag{2.10}$$

where the tilde (~) states for median. Both the median – as a location statistic – and the MAD – as a scale statistic – are very robust summary statistics and I found them adequate to summarize individual behaviour in the face of the diversity of distributions I found in the behavioural features (Figure 2.12).

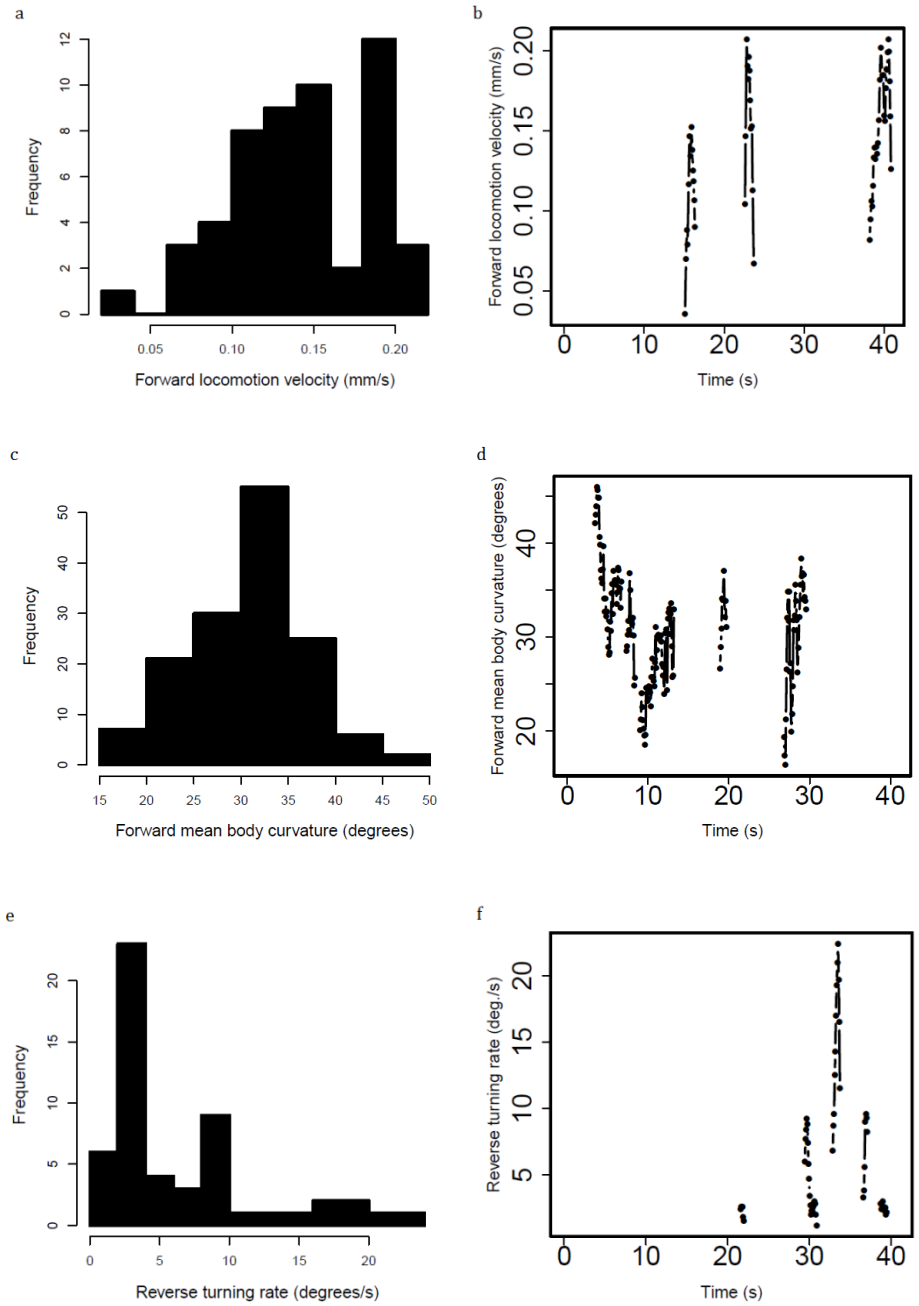


Figure 2.12 – The diversity of distributions among the behavioural features tracked (a, c, e), with the respective temporal dynamics (b, d, f). Three behavioural features are shown. a, b. Forward locomotion velocity

(FwdVelocity). c, d. Mean body curvature (Curvature). e, f. Reverse turning rate (RevTurningRate).

We can divide the behavioural features in three classes, which are distinguished by the scale at which they are measured. In a first class, we have state-based features, measured in every frame of tracking, which then give rise to time-series – examples are velocity and turning rate. In a second class, we have behaviours based on events, which can occur only in some of the frames of tracking, e.g. forward bout duration, interval between bouts. These features are only quantified if they occur and in these the median and MAD are calculated as the median and MAD of all events during the tracking time. If a given event-based behaviour does not occur, it is considered missing data. A third class of behaviours is cumulative-based, in which behaviour is only quantified based on the whole track; as a consequence, these behaviours display only one value per worm track, e.g. exploration rate and distance to average neighbour. In these, there is only one value per track, which will work as a median; in these, MAD cannot be calculated for there is no scale summary statistic that can be applied to a single value.

Given the intervals I have chosen to extract the worm tracks from, in many occasions I have obtained several worm tracks belonging to the same worm, i.e. from the same worm ID. For these reasons, besides having a worm track median and MAD, I have also calculated the average both of these statistics at the level of the worm by averaging the several worm tracks from the same worm.

Ensuring statistical independence among nematode tracks under analysis

The MWT is able to track hundreds of nematodes simultaneously, yet individual tracks are lost when nematodes interact physically and, if they separate again, the track identity of each nematode cannot be traced back to the period preceding the interaction. This means we may have several tracks of the same nematode separated by a very short time interval. As I work here with summary statistics of the nematode tracks, the use of correlated tracks in time would give rise to correlated summary statistics within each inbred line or population. Therefore, I needed a strategy to avoid using correlated tracks of the same individuals for analysis. This strategy was divided in two stages: in the first stage, I have determined the minimum period that ensures representative summary statistics for each individual nematode track and and in the second stage I have determined the interval between sampling periods that ensures independence of nematode tracks.

For the first stage, I have randomly selected 100,000 tracks more than 3 minutes long – i.e. 180 seconds – coming from all inbred lines and defined the minimum track sampling time as the minimum tracking time at which behaviour median absolute deviation from the median (MAD) has reached a stable value. When this stable value is reached, an increase in length of track samples would not change significantly the MAD obtained from that nematode track. Two features were under analysis here, length (Table 2.2; Figure 2.9) and velocity (Table 2.1; Figure 2.5). I have randomly sub-sampled ten tracks of increasing lengths – from 1 to 110 seconds – from each nematode track, then I have fitted a model to each of the features – length and velocity – and then took its derivative. The tracking time each nematode track should have for further analysis was dictated by the minimum tracking time at which this derivative reached

zero. Given the MAD dynamics I have observed as a function of sampling time, I have applied a stepwise regression model, starting with a linear term, a logarithmic term and a square root term,

$$s = b_0 + b_1 p + b_2 \log p + b_3 \sqrt{p} \tag{2.11}$$

In this model, s is the MAD in question, p is the nematode track length (persistence, as defined in Table 2.2) and b_k are the effects of each term k to be estimated in the regression. In the stepwise procedure, one regression term is removed at a time and a new regression model is done without that term and compared to the previous model; this process is known as backward elimination. The aim is to find the best model possible given our specified starting model; in this case, we start with three terms and we aim to find the best model by reducing the number of terms. The quality of the model is assessed by the amount of information lost between the model I use and the real data, and that amount was measured using the Akaike Information Criterion (AIC; Akaike, 1998; Mallows, 1973),

$$AIC = n \log\left(\frac{1}{n}\sum_{i=1}^{n}(s_i - \widehat{s_i})^2\right) + 2k \tag{2.12}$$

In this equation, n is the sample size – the number of data points –, s_i is the i-th measurement MAD (length or velocity), $\widehat{s_i}$ is the prediction of the same i-th MAD given by the model under analysis and k the number of estimated parameters in the model. The lower the AIC, the better the

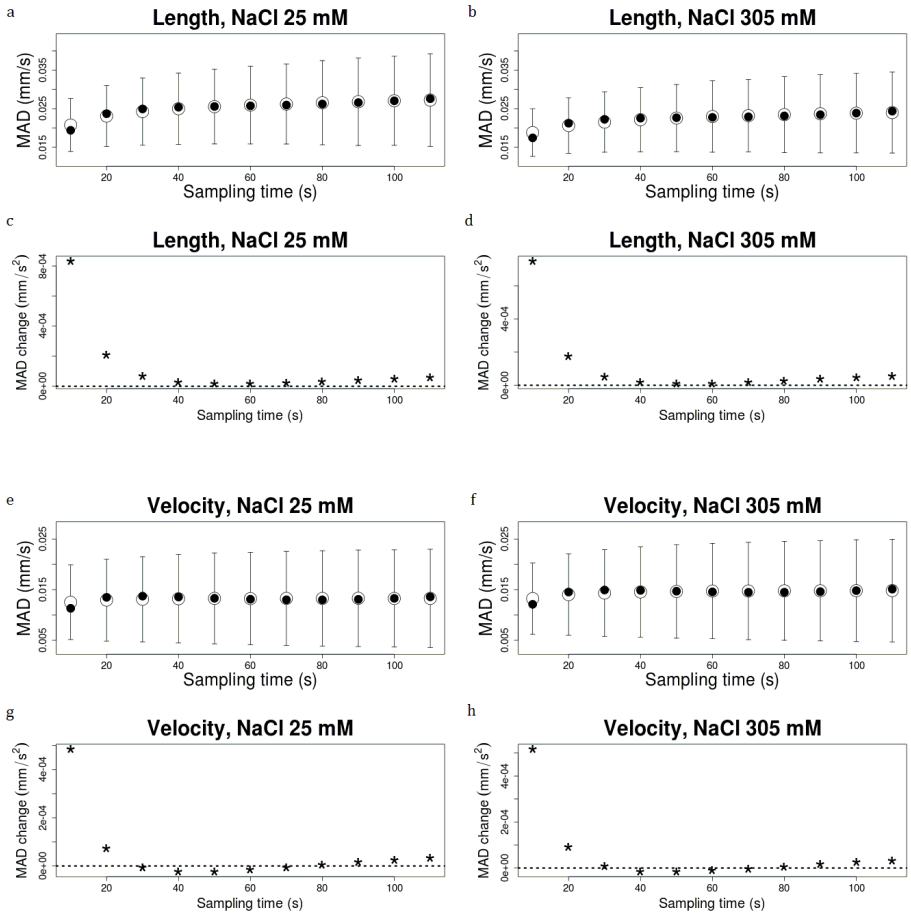
model should be. The first term represents the quality of model fit, for it takes into account the squared difference between values predicted by the model and the observed values, i.e. the squared residuals; actually, the term inside the logarithm is the residual sum of squares. The second term is a penalty applied to the model due to its complexity, i.e. due to the number of parameters used to explain the data. For instance, in Equation 2.3 there are three parameters, so k=3. In summary, AIC assesses the quality of a model both by the quality of its fitting to the data and by the number of required parameters for that model fit.

For the second stage, I have used the same randomly selected tracks previously mentioned analysed the autocorrelation structure within each nematode track in time on two measurements, one morphological – length (see Table 2.2) – and one behavioural – velocity (see Table 2.1) –, using the 'acf' function in R (R Core Team, 2014). From this analysis, I have defined the interval between sampling periods that would guarantee independent tracks as the maximum time, among measurements and environments, at which behavioural autocorrelation reached zero. The autocorrelation of a variable is a correlation between values of a variable separated by a specific time lag (Box et al., 1994). Being τ the time lag between the values of a given variable x, autocorrelation was here defined as

$$R(\tau) = \frac{\sum_{i=1}^{n}\left(x_{t,i} - \bar{x}\right)\left(x_{t+\tau,i} - \bar{x}\right)}{s_x} \qquad (2.13)$$

where x_t is the variable value in times t, $x_{t+\tau}$ are x values in times $t+\tau$, $\bar{x}$ the variable mean and s_x its overall standard deviation.

In the first stage of analysis, I have found that length MAD values start to stabilize at around 40 seconds and velocity values start stabilizing at 30 seconds in both environments (Figure 2.13a,b), as derivatives of the fitted functions stabilize at zero in these sampling times (Figure 2.13c,d). Therefore, I have considered 40 seconds as a minimum track duration needed to obtain stable values. In the second stage of analysis, I found that for both length and velocity autocorrelation drops to near zero values in around 70 seconds and starts flattening, reaching a stable zero autocorrelation at around 80 seconds (Figure 2.13e,f). Therefore, I have decided to use an interval of 80 seconds between track samples.



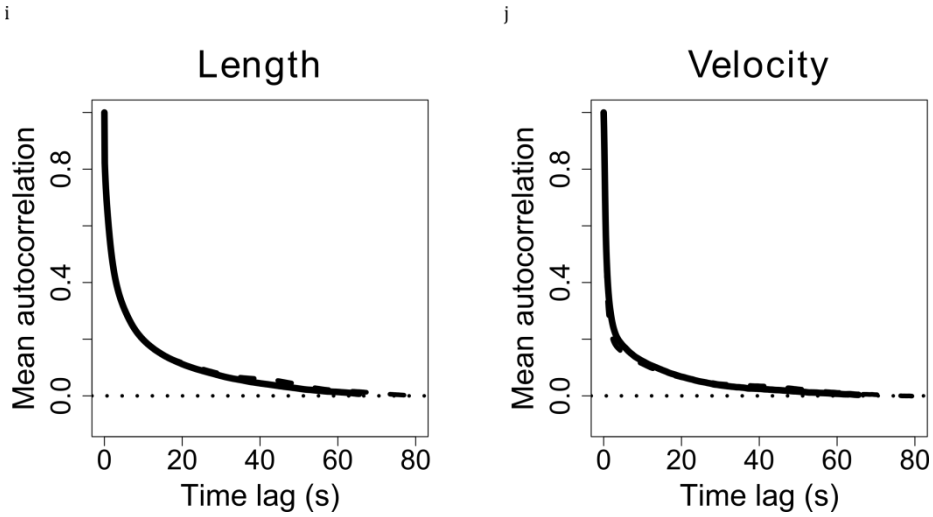


Figure 2.13 – Statistical independence is achieved with worm tracks at least 40 seconds long, with 80-second intervals in between. Behaviour of a given individual in different tracks will be independent among these intervals, even if the same individual is captured in two tracks in consecutive time periods. For this analysis, I have used 100,000 worm tracks at least 180 seconds long which were randomly sampled from all inbred lines. Length MAD in NaCl 25 mM (**a**) and NaCl 305 mM (**b**) increases asymptotically with worm track duration and starts to stabilize when tracks are at least 40 seconds long. **c,d.** Derivatives of the length MAD function in NaCl 25 mM (**c**) and 305 mM (**d**) with regards to worm track persistence (p in Equation 2.3), took from the best models shown in **a** and **b**, respectively. Both derivatives stabilize at around zero from 40 seconds on. This means that for a stable estimate of length MAD, I need tracks at least 40 seconds long. e,f. Velocity MAD also increases asymptotically, stabilizing actually at around 30 seconds both in NaCl 25 mM (**e**) and in NaCl 305 mM (**f**). In **a, b, e** and **f**, white points represent observed values with standard deviations as error bars, and black points are the best model fitted values. The best model equations are stated in Appendix 1. **g,h.** Velocity function derivatives with regards to worm track persistence in NaCl 25 mM (**g**) and NaCl 305 mM (**h**), took from the best models shown in **e** and **f**, respectively. Velocity derivatives stabilize at around zero from 30-second tracks on in both environments. **i, j.** Autocorrelation function for length (**i**) and velocity (**j**) using the same worm tracks.

Autocorrelation profiles start approaching to zero at around 70 seconds both in NaCl 305 mM (filled lines) and in NaCl 25 mM (dashed lines). Therefore, an interval of 80 seconds was needed between samples.

In summary, I have obtained a minimum worm track sampling time of 40 seconds and a necessary interval of 80 seconds between measurements within the same worm in order to reach stable MAD values and replicate independence within a given worm (Figure 2.13). I therefore established twelve 40-to-41-second time periods evenly spaced by 80 seconds, from which I have extracted nematode tracks at least 40 seconds long. As a result, I have ended up with nematode tracks of lengths between 40 and 41 seconds. I have also excluded the first 100 seconds of tracking in order to avoid analysing behavioural responses to the mechanical stimuli that might have arisen from Petri plate handling before tracking. Therefore, the intervals used to gather the tracks are represented here in seconds as]100,141],]221,262],]342,383],]463,504],]584,625],]705,746],]826,867],]947,988],]1068,1109],]1189,1230],]1310,1351],]1431,1472].

Taking advantage of inbred line fecundity measurements

I took advantage of data obtained by Ivo M. Chelo, Alessa Silva and Fredilson Melo on fecundity of some of the inbred lines, both from the ancestral population 140A6 and from the evolved populations GA1 and GA2. After reviving, individuals of each inbred line were kept in NaCl 25 mM for two generations. On the third generation after reviving, individual nematodes were raised in each of two environments, NGM 25 mM NaCl

and NGM 305 mM NaCl in regular 90 mm Petri dishes, in the same density of the experimental evolution (~1,000 individuals per plate) until the fourth larval stage (L4). At this stage, individual hermaphrodites were transferred to individual wells of 96-well plates, each of which with 100 µL NGM 25 mM NaCl or NGM 305 mM NaCl, respectively and both with 1 µL *E. coli* HT115 grown overnight to a density of 10^7-10^8 cells. After 24 hours at 20 °C and 80% relative humidity, these hermaphrodites have undergone a mixed treatment of M9 solution and sodium hypochlorite 5% (1:1) for 5 minutes, which sacrificed the adult hermaphrodites but kept the eggs. Then, 200 µL of M9 liquid solution were added and rinsed 3 times. Then, this liquid was transferred to another 96-well plate, which had been previously filled with 120 µl of M9 for a second rinsing step. Finally, plates were centrifuged for one minute at 652 rcf and left overnight at 20 °C and 80% relative humidity. After 24 hours of incubation, pictures were taken with 2x objective with Nikon Eclipse TE2000-S and the number of L1 larvae was counted manually from these pictures.

Estimating selection gradients on behaviour centrality and behaviour variability

I have estimated selection pressures acting on the ancestral population using the inbred lines derived from the ancestral population by using measurements of fecundity and behaviour. Specifically, I have used the number of live L1 larvae resulting from each hermaphrodite egg-laying as a proxy for fitness. To obtain phenotypic selection gradients estimated as referred in the introduction, we would need to have measured both

behaviour and fitness in all individuals, which was technically unfeasible. What I have here are mean fecundity estimates for each inbred line, on one hand, and mean behaviour feature estimates for each inbred line, on the other hand. These data allow estimation of linear, directional selection pressures B and also of quadratic selection pressures Γ, which are, respectively, the linear and quadratic selection coefficients on the family means of the behavioural features I have analysed (Rausher, 1992), each family being one inbred line. Inbred lines behavioural feature means were standardized prior to regression so that the overall inbred line feature mean is 0, *i.e.* $\bar{\tilde{z}}_i = 0$ for all traits i (see Equations 1.3 and 1.4). Also, relative fitness proxies were obtained by dividing the fecundities of each inbred line by the mean fecundity in the respective environment.

On a first, univariate approach, a quadratic approximation of the individual selection surface on inbred line means was fitted using each behavioural feature and this relative fitness proxy. Because most inbred line behaviour means present deviations from normality (not shown), linear and quadratic selection gradients were calculated separately (see Equations 1.1 and 1.2) and statistical significance was assessed using a permuted data distribution generated by doing 2,000 random permutations of the data.

Then, to compare behaviour and behaviour variation in terms of strength of selection, selection gradients are not enough because they do not take the scale of measurements into account. This comparison is possible using elasticities of selection, which are selection gradients standardized by the phenotypic mean (Morgan and Schoen, 1997; van Tienderen, 2000). This standardization has the advantage of taking the scale of phenotypes into account and being largely independent of the population phenotypic variance, contrasting with other standardizations such as the standardization by the standard deviation done in the intensity of

selection (Falconer, 1960), which is obtained after multiplying gradients by the standard deviation, being therefore dependent on the phenotypic variance. The linear elasticity of selection is the linear selection gradient multiplied by the population mean (Hereford et al., 2004; Matsumura et al., 2012),

$$B_{\mu} = \bar{z}B \tag{2.14}$$

whereas the quadratic elasticity of selection is the multiplication of the quadratic selection gradient by the squared of the population mean (Hereford et al., 2004),

$$\Gamma_{\mu} = \bar{z}^2\Gamma. \tag{2.15}$$

The original notation uses the selection gradients β and curvatures γ, but here it is replaced by the pressures B and Γ, the ones effectively estimated.

In a second approach, multivariate, all behavioural features, including both median and MAD measurements, were included in a linear approximation of the selection surface. In other words, all these traits were included in a multiple regression in the form described in equation 2.7. Performing a quadratic approximation of the selection surface using 81 behavioural traits encompassing median and MAD values would imply estimating a total of 3483 terms – 81 linear terms and 3402 quadratic terms, including 81 quadratic terms of a single trait and 3321 quadratic terms regarding correlational selection. As this number vastly exceeds

the number of inbred lines available, I have performed a linear approximation of the selection surface, which implies the estimation of the 81 linear terms. Nevertheless, this estimation of directional selection pressures serves the purpose of testing selection on behaviour and behaviour variability and this selection can eventually lead to a change in population mean of the respective features. The inclusion of both median and MAD measurements in the same selection surface allows addressing a selection on behaviour and behaviour variability and accounting for effects of scale on behaviour variability. By accounting for these effects of scale, one could say that selection on behaviour variability in a given trait was not detected because of selection on the median of any other trait (Lynch and Walsh, 1998), in the same way one can distinguish between direct selection on a trait and selection on a correlated trait (Lande and Arnold, 1983; Pearson, 1903). I have used this multiple regression model as a starting model, because even this 81-term linear model would likely have too many parameters. The best model with only linear terms was obtained by stepwise regression from this starting model to a neutral model, without any term – in which no feature would be relevant for fitness. To assess the quality of each model, the Akaike Information Criterion (AIC; equation 2.4) was used, being the best model the one with the lowest AIC. The stepwise procedure started with the AIC calculation of the starting model. Then, reduced models with one less term are performed and their AIC is compared with the full model's AIC. If the AIC of this reduced model is lower than the one of the full model, then this reduced model is taken as a reference model and a second reduced model, with two less features than the full model, is applied. All reduced models with two less features are compared to our reference model - with one less feature than the full model. The reduced model with the lowest AIC will be then adopted as the reference model. After a certain point, when the full model has been reduced in several features, this

model comparison process can go back and forth - by re-adding one and removing one feature at a time -, until the AIC reaches a minimum that is not lowered by either addition or subtraction of any other feature.

Because the best linear approximation of the selection surface still included an appreciable number of terms, I have addressed the possibility of multicollinearity, which would arise if there are highly correlated terms in the regression in such a way that the effects of a given trait on fitness could be predicted from effects of other traits. These terms would be collinear; estimation of effects of any term belonging to a group of collinear terms is not possible because it is not possible to observe changes in a value of one of the terms without observing concomitant changes in the values of other terms (Mitchell-Olds and Shaw, 1987). Variance estimates of the collinear terms tend to increase relative to a case when there are no collinear terms, because these estimates also include shared variance among the collinear terms. If R_i^2 is the proportion of variance in the term *i* that is associated with the other terms in the regression, then the variance inflation factor (VIF) for *i* is defined as below,

$$VIF_i = \frac{1}{1 - R_i^2} \tag{2.16}$$

in which the denominator is called the tolerance for the term *i* and represents the amount of variance on this term that is not related to other regression terms (O'Brien, 2007). I have then calculated the VIF for each term of all models in which I addressed the multicollinearity issue.

Spotting a multicollinearity issue given VIF values is not under agreement along statisticians, however I considered here VIFs higher than 3 as a

matter of concern (Zuur et al., 2010) and VIFs higher than 10 as events of serious multicollinearity that demanded a revision on the model to be used, in accordance to statistical recommendations (Menard, 1995; Neter et al., 1989).

Elasticities of selection on median and MAD were compared using the Welch's *t* test. This *t* test compares group means and is also applicable when the statistical groups under comparison have different variances (Welch, 1947). For this comparison, only the state and event-based features defined above were used, because these were the only ones that had both median and MAD values. For instance, forward velocity (state-based) and forward bout duration (event based) have both a median and a MAD for analysis and they were thus used, as opposed to exploration rate, which was not used. For comparison of univariate quadratic selection gradients, a square-root transformation was applied to the coefficients in order to approximate the distribution of the coefficients to normality.

Principal components analyses on behaviour centrality and variabilty for reduction of multicollinearity in the multivariate selection surfaces

When the multivariate selection surface presented issues of multicollinearity, I have applied principal components analysis (PCA) separately on the family means of the behaviour medians and on behaviour MADs and repeated the multivariate selection surface on these principal components. Principal components analysis is a technique that allows us to rotate the data and into an orthogonal space, with as many

dimensions as the original space, but with features defined by descending variances. Each of these new features are called principal components and is obtained as a linear combination of all the original features and the components resulting from a given PCA are by definition linearly independent (Jolliffe, 2005). I have derived the principal components by spectral decomposition of the covariance matrix of the family means. The linear combinations that describe each feature lie in the eigenvectors of the covariance matrix and the principal component values X were obtained by rotation of the original family means. This rotation results from matrix multiplication between the original family mean A matrix and the matrix of eigenvectors v,

$$X = Av. \tag{2.17}$$

This approach, on one hand, aimed to rotate the behaviour family mean data into a nearly orthogonal space, which would decrease multicollinearity. The decrease of multicollinearity would allow me to interpret a selection coefficient on a given trait as respective to that trait and not also to other traits included in the regression (Mitchell-Olds and Shaw, 1987). On the other hand, by performing the principal components analysis separately in the behaviour medians and MADs, I aimed to obtain components that only referred to medians and MADs and that would allow me to check directly for selection on median and on MAD components, respectively.

Measuring the influence of covariates on the behaviour statistics

I have used linear mixed models to assess how covariates have changed with generation of populations and inbred lines under tracking. This approach aimed to assess the need to take these covariates into account when assessing the evolutionary reponses of behavioural features. The linear mixed models included each covariate – number of individuals on the tracking plate, temperature and relative humidity at the end of tracking, M9 volume in the generations of tracking and the one previous to tracking – separately as a response and took the respective forms below for populations (index o) and inbred lines (index i),

$$c_o = Generation + Block + Population + e_o \tag{2.18}$$

$$\begin{gathered} c_i = \\ Generation + Block + Population + \\ +Inbred\{Population\} + e_i. \end{gathered} \tag{2.19}$$

In these models, generation was the only fixed effect and all other effects were random. The effect estimates were obtained through planned contrasts of least-squares means (Lenth, 2016) of covariate values at each generation; degrees of freedom were calculated using the Kenward-Roger approximation (Halekoh and Højsgaard, 2014). The models were applied separately in the two environments. Both in populations and inbred lines, in both environments, no significant effect was found for any covariate along generations (Tables 2.4 – 2.7). This means it is very unlikely that differences in covariates along generations can explain evolutionary responses observed in any behavioural feature.

Table 2.4 – Estimates of the effects of generation on the covariates measured in the populations, in NaCl 25 mM. Covariates are described in the text. S.E., standard error; d.f., degrees of freedom.

	Estimate	**S.E.**	**T**	***d.f.***	***P***
Temperature	0.209271	0.171505	1.220207	4.918444	0.277641
Relative humidity	-0.55673	1.3767	-0.40439	4.805604	0.703293
M9 volume (Previous to tracking)	-5.990150	21.57886	-0.27759	2.145202	0.805812
M9vol (Tracking)	13.51075	16.62936	0.812464	2.133382	0.497201
Number of individuals	-6.500000	54.30989	-0.11968	1.994573	0.915699

Table 2.5 – Estimates of the effects of generation on the covariates measured in the populations, in NaCl 305 mM. Covariates are described in the text. S.E., standard error; d.f., degrees of freedom.

	Estimate	**S.E.**	**T**	***d.f.***	***P***
Temperature	-0.00947	0.284	-0.03341	3.332601	0.975249
Relative humidity	0.1158930	1.454776	0.079664	3.957343	0.94037
M9 volume (Previous to tracking)	-5.990150	21.57886	-0.27759	2.145202	0.805812
M9vol (Tracking)	13.51075	16.62936	0.812464	2.133382	0.497201
Number of individuals	4.597303	72.51137	0.063401	1.715585	0.956042

Table 2.6 – Estimates of the effects of generation on the covariates measured in the inbred lines, in NaCl 25 mM. Covariates are described in the text. S.E., standard error; d.f., degrees of freedom.

	Estimate	**S.E.**	**T**	***d.f.***	***P***
Temperature	-0.025330	0.044641	-0.56743	0.468499	0.737559
Relative humidity	0.0739610	0.269011	0.274936	0.466959	0.859937
M9 volume (Previous to tracking)	11.13329	2.813782	3.956701	0.445	0.35247
M9vol (Tracking)	10.96984	2.350335	4.66735	0.443	0.329384
Number of individuals	-77.68620	23.61422	-3.28981	0.940	0.200472

Table 2.7 – Estimates of the effects of generation on the covariates measured in the inbred lines, in NaCl 305 mM. Covariates are described in the text. S.E., standard error; d.f., degrees of freedom.

	Estimate	**S.E.**	**T**	***d.f.***	***P***
Temperature	-0.03783	0.07634	-0.49551	1.086178	0.701791
Relative humidity	0.22974	0.25334	0.906829	0.442974	0.643142
M9 volume (Previous to tracking)	11.09734	2.813032	3.944975	0.445717	0.352558
M9vol (Tracking)	11.02039	2.349829	4.689867	0.44404	0.327802
Number of individuals	-66.51900	24.92217	-2.66907	0.978744	0.23256

Assessing evolution of behaviour and behaviour variability

I have used linear mixed models of both worm track behaviour medians and MADs to assess, respectively, evolution of behaviour and behaviour variability on each feature. Each linear mixed model was applied separately in each environment and took the forms below in the populations and inbred lines, respectively,

$$y_o = Generation + Block + Population + \\ + Replicate\{Population\} + e_o \tag{2.20}$$

$$y_i = Generation + Block + Population + \\ + Inbred\{Population\} + \\ + Replicate\{\{Inbred\{Population\}\} + e_i \tag{2.21}$$

in which *e* are the residuals of the respective models. The subscripts *o* and *i* emphasize the reference to populations (outbred) and inbred lines, respectively. I have assessed evolution as response to selection by estimating the effect of generation from the linear mixed models on each feature. In order to minimize deviations from normality in residual distribution and heterogeneity in the inbred line variances among individuals, a diverse set of transformations was applied to both behaviour centrality features (Table 2.8) and behaviour variability features (Table 2.9). Statistical significance of the evolutionary responses was assessed using *t* tests.

Table 2.8 – Transformations applied to the behaviour centrality (median) features. Numbers in parentheses are the values of λ used for Box-Cox transformation (Box and Cox, 1964; Hyndman et al., 2015) of the respective feature.

Transformation	**Features**
None	Regressed length, curvature, forward curvature, reverse curvature, stationary curvature, forward fraction, reverse fraction, reverse distance, locomotion fraction, net distance travelled
Logarithmic $x' = \log(x + 1)$	Turning rate, forward turning rate, forward duration, reverse turning rate, reverse duration, stationary turning rate, exploration density, forward distance, total distance travelled
Square-root $x' = \sqrt{x + 1}$	Reverse bout frequency, forward bout frequency, interval between reverse bouts,
Box-Cox $x' = \frac{x^{\lambda} - 1}{\lambda}$	Aspect (−0.4075424), regressed width (−0.07080766), velocity (−0.5979633), forward regressed length (1.191195), forward regressed

	width (−0.04472859), forward longitudinal bending (−0.9999242), forward lateral bending (0.03748749), forward aspect (−0.3715418), forward velocity (−0.5618042), reverse regressed length (1.126253), reverse regressed width (0.06860481), reverse longitudinal bending (−0.9999242), reverse lateral bending (0.1482776), reverse aspect (−0.2120651), reverse velocity (−0.2504979), stationary regressed length (1.381544), stationary regressed width (−0.1141212), stationary longitudinal bending (−0.9999242), stationary lateral bending (0.03805558), stationary aspect (−0.4368298), interval between forward locomotion bouts (−0.1934558), interval between locomotion bouts (−0. 1934558), exploration rate (−0.07123803), distance nearest average neighbour (0.01665557), distance nearest average neighbour standardized (0.0543301)

Table 2.9 – Transformations applied to the behaviour variability (MAD) features. Numbers in parentheses are the values of λ used for Box-Cox transformation (Box and Cox, 1964; Hyndman et al., 2015) of the respective feature.

Transformation	**Features**
None	Reverse aspect, forward aspect, forward velocity, forward regressed length, reverse

	regressed width, reverse duration, reverse interval, forward longitudinal bending, reverse longitudinal bending, forward regressed width, reverse duration
Logarithmic $x' = \log(x + 1)$	forward interval, forward duration, bout interval
Square-root $x' = \sqrt{x + 1}$	Curvature, forward curvature, reverse curvature
Box-Cox $x' = \frac{x^{\lambda} - 1}{\lambda}$	Velocity (−0.3181043), turning rate (−0.1665744), regressed length (−0.128696), regressed width (0.1254512), aspect (−0.09122768), reverse lateral bending (0.1898755), forward lateral bending (0.3194302), reverse velocity (0.1236343), stationary regressed width (0.08999245), stationary longitudinal bending (−0.2311582), stationary lateral bending (0.04228231), stationary aspect (−0.1132957), stationary curvature (−0.1607806), forward turning rate (−0.1665744), reverse turning rate (−0.1665744), stationary regressed length (−0.128696), stationary turning rate (−0.1665744)

Estimating broad-sense heritability in behaviour centrality and behaviour variability features

As mentioned in the previous chapter, broad-sense heritability is the fraction of phenotypic variance that has a genetic origin. Here, the inbred lines derived from the ancestral population were used to estimate that

fraction. For each environment, a variance components model was used for each feature – centrality (median) and variability (MAD) – that had the form below,

$$V_P = V_{Line} + V_{Replicate\{Line\}} + V_{Block} + V_e \quad (2.22)$$

in which V_P is the variance observed in each feature – the phenotypic variance –, V_{Line} is the variance among inbred lines, $V_{Replicate\{Line\}}$ is the variance among replicates within each inbred line (thus nested within inbred line in the variance components model), V_{Block} is the variance among experimental blocks and V_e is the residual variance. In this model, the genetic variance component is captured by V_{Line} and several environmental components are captured by the remaining variance components. Because we are dealing with inbred lines derived from the population and not the population itself, the genetic variance (V_G in Equation 1.5) is half the variance among inbred lines (Falconer and Mackay, 1995; Phillips, 1998) and thus broad-sense heritability was estimated as

$$H^2 = \frac{V_{Line}}{2\left(V_{Line} + V_{Replicate\{Line\}} + V_{Block} + V_e\right)} \quad (2.23)$$

and statistical significance of these estimates was assessed using 100 bootstrap replicates of each feature. The p-value reported was the fraction of bootstrap estimates that brought a heritability estimate higher than the one brought by the original data.

References

Akaike, H. (1998). Information Theory and an Extension of the Maximum Likelihood Principle. In Selected Papers of Hirotugu Akaike, E. Parzen, K. Tanabe, and G. Kitagawa, eds. (Springer New York), pp. 199–213.

Altun, Z.F., and Hall, D.H. (2002). Handbook of *C. elegans* anatomy. In *WormAtlas*.

Barrett, R.D.H., and Schluter, D. (2008). Adaptation from standing genetic variation. Trends Ecol. Evol. *23*, 38–44.

Bendesky, A., Tsunozaki, M., Rockman, M.V., Kruglyak, L., and Bargmann, C.I. (2011). Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. Nature *472*, 313–318.

Box, G.E.P., and Cox, D.R. (1964). An Analysis of Transformations. J. R. Stat. Soc. Ser. B Methodol. *26*, 211–252.

Box, G.E.P., Jenkins, G.M., and Reinsel, G.C. (1994). Time series analysis: forecasting and control (Prentice Hall).

Byerly, L., Cassada, R.C., and Russell, R.L. (1976). The life cycle of the nematode *Caenorhabditis elegans*: I. Wild-type growth and reproduction. Dev. Biol. *51*, 23–33.

Chelo, I.M., and Teotónio, H. (2013). The Opportunity for Balancing Selection in Experimental Populations of *Caenorhabditis elegans*. Evolution *67*, 142–156.

Cronin, C.J., Mendel, J.E., Mukhtar, S., Kim, Y.-M., Stirbl, R.C., Bruck, J., and Sternberg, P.W. (2005). An automated system for measuring parameters of nematode sinusoidal movement. BMC Genet. *6*, 5.

Crow, J.F., and Kimura, M. (1970). An introduction to population genetics theory. xiv+591 pp..

Falconer, D.S. (1960). Introduction to Quantitative Genetics (Longman).

Falconer, D.S., and Mackay, T.F.C. (1995). Introduction to Quantitative Genetics (Essex, England: Longman).

Feng, Z., Cronin, C.J., Wittig, J.H., Sternberg, P.W., and Schafer, W.R. (2004). An imaging system for standardized quantitative analysis of *C. elegans* behavior. BMC Bioinformatics *5*, 115.

Frazier, H.N., and Roth, M.B. (2009). Adaptive Sugar Provisioning Controls Survival of *C. elegans* Embryos in Adverse Environments. Curr. Biol. *19*, 859–863.

Galea, J.M., Ruge, D., Buijink, A., Bestmann, S., and Rothwell, J.C. (2013). Punishment-Induced Behavioral and Neurophysiological Variability Reveals Dopamine-Dependent Selection of Kinematic Movement Parameters. J. Neurosci. *33*, 3981–3988.

Gray, J.C., and Cutter, A.D. (2014). Mainstreaming *Caenorhabditis elegans* in experimental evolution. Proc. R. Soc. Lond. B Biol. Sci. *281*, 20133055.

Halekoh, U., and Højsgaard, S. (2014). A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models. J. Stat. Softw. *59*, 1–32.

Hereford, J., Hansen, T.F., and Houle, D. (2004). Comparing Strengths of Directional Selection: How Strong Is Strong? Evolution *58*, 2133–2143.

Hill, W.G. (1982). Rates of change in quantitative traits from fixation of new mutations. Proc. Natl. Acad. Sci. *79*, 142–145.

Hope, I.A. (2000). *C. elegans*: A Practical Approach (Oxford University Press).

Hyndman, R.J., Athanasopoulos, G., Bergmeir, C., Cinelli, C., Khan, Y., Mayer, Z., Razbash, S., Schmidt, D., Shaub, D., Tang, Y., et al. (2015). forecast: Forecasting Functions for Time Series and Linear Models. R package version 6.2.

Jarrell, T.A., Wang, Y., Bloniarz, A.E., Brittin, C.A., Xu, M., Thomson, J.N., Albertson, D.G., Hall, D.H., and Emmons, S.W. (2012). The Connectome of a Decision-Making Neural Network. Science *337*, 437–444.

Jolliffe, I. (2005). Principal Component Analysis. In Encyclopedia of Statistics in Behavioral Science, (John Wiley & Sons, Ltd).

Lamitina, S.T., Morrison, R., Moeckel, G.W., and Strange, K. (2004). Adaptation of the nematode *Caenorhabditis elegans* to extreme osmotic stress. Am. J. Physiol. - Cell Physiol. *286*, C785–C791.

Lande, R., and Arnold, S.J. (1983). The Measurement of Selection on Correlated Characters. Evolution *37*, 1210.

Lenth, R. (2016). lsmeans: Least-Squares Means. R package version 2.23.

Lynch, M., and Walsh, B. (1998). Genetics and Analysis of Quantitative Traits (Sunderland, Mass.: Sinauer Associates Inc.,U.S.).

Mallows, C.L. (1973). Some Comments on C_p. Technometrics *15*, 661–675.

Matsumura, S., Arlinghaus, R., and Dieckmann, U. (2012). Standardizing Selection Strengths to Study Selection in the Wild: A Critical Comparison and Suggestions for the Future. BioScience *62*, 1039–1054.

Menard, S. (1995). Applied Logistic Regression Analysis (Thousand Oaks, CA, USA: Sage Publications, Inc.).

Mitchell-Olds, T., and Shaw, R.G. (1987). Regression Analysis of Natural Selection: Statistical Inference and Biological Interpretation. Evolution *41*, 1149–1161.

Morgan, M., and Schoen, D. (1997). Heredity - Abstract of article: Selection on reproductive characters: floral morphology in *Asclepias syriaca*. Heredity *79*, 433–441.

Neter, J., Wasserman, W., and Kutner, M.H. (1989). Applied Linear Regression Models (Homewood, IL, USA: Irwin Professional Publishing).

O'Brien, R.M. (2007). A Caution Regarding Rules of Thumb for Variance Inflation Factors. Qual. Quant. *41*, 673–690.

Ölveczky, B.P., Andalman, A.S., and Fee, M.S. (2005). Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol *3*, e153.

Pearson, K. (1903). Mathematical Contributions to the Theory of Evolution. XI. On the Influence of Natural Selection on the Variability and Correlation of Organs. Philos. Trans. R. Soc. Lond. Ser. Contain. Pap. Math. Phys. Character *200*, 1–66.

Phillips, P.C. (1998). H2boot: bootstrap estimates and tests of quantitative genetic data (University of Oregon). Software available in www.uoregon.edu/pphil/software.html.

R Core Team (2014). R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

Ramot, D., Johnson, B.E., Berry, T.L., Jr, Carnell, L., and Goodman, M.B. (2008). The Parallel Worm Tracker: A Platform for Measuring Average Speed and Drug-Induced Paralysis in Nematodes. PLoS ONE *3*, e2208.

Rausher, M.D. (1992). The Measurement of Selection on Quantitative Traits: Biases Due to Environmental Covariances between Traits and Fitness. Evolution *46*, 616–626.

Rowlingson, B., and Diggle, P. (2013). splancs: Spatial and Space-Time Point Pattern Analysis. R package version 2.01-34.

Shingai, R. (2000). Durations and frequencies of free locomotion in wild type and GABAergic mutants of *Caenorhabditis elegans*. Neurosci. Res. *38*, 71–84.

Shtonda, B.B., and Avery, L. (2006). Dietary choice behavior in *Caenorhabditis elegans*. J. Exp. Biol. *209*, 89–102.

Solomon, A., Bandhakavi, S., Jabbar, S., Shah, R., Beitel, G.J., and Morimoto, R.I. (2004). *Caenorhabditis elegans* OSR-1 Regulates Behavioral and Physiological Responses to Hyperosmotic Environments. Genetics *167*, 161–170.

Stephens, G.J., Johnson-Kerner, B., Bialek, W., and Ryu, W.S. (2008). Dimensionality and Dynamics in the Behavior of *C. elegans*. PLoS Comput Biol *4*, e1000028.

Stiernagle, T. (2006). Maintenance of *C. elegans*. WormBook.

Swierczek, N.A., Giles, A.C., Rankin, C.H., and Kerr, R.A. (2011). High-throughput behavioral analysis in *C. elegans*. Nat. Methods *8*, 592–598.

Tauffenberger, A., and Parker, J.A. (2014). Heritable Transmission of Stress Resistance by High Dietary Glucose in *Caenorhabditis elegans*. PLoS Genet *10*, e1004346.

Teotonio, H., Carvalho, S., Manoel, D., Roque, M., and Chelo, I.M. (2012). Evolution of Outcrossing in Experimental Populations of *Caenorhabditis elegans*. PLoS ONE *7*, e35811.

Theologidis, I., Chelo, I.M., Goy, C., and Teotónio, H. (2014). Reproductive assurance drives transitions to self-fertilization in experimental *Caenorhabditis elegans*. BMC Biol. *12*, 93.

van Tienderen, P.H. (2000). Elasticities and the link between demographic and evolutionary dynamics. Ecology *81*, 666–679.

Tsibidis, G.D., and Tavernarakis, N. (2007). Nemo: a computational tool for analyzing nematode locomotion. BMC Neurosci. *8*, 86.

Welch, B.L. (1947). The Generalization of "Student"s' Problem When Several Different Population Varlances Are Involved. Biometrika *34*, 28–35.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The Structure of the Nervous System of the Nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. B Biol. Sci. *314*, 1–340.

Wu, H.G., Miyamoto, Y.R., Castro, L.N.G., Ölveczky, B.P., and Smith, M.A. (2014). Temporal structure of motor variability is dynamically regulated and predicts motor learning ability. Nat. Neurosci. *17*, 312–321.

Yamada, K., Hirotsu, T., Matsuki, M., Butcher, R.A., Tomioka, M., Ishihara, T., Clardy, J., Kunitomo, H., and Iino, Y. (2010). Olfactory Plasticity Is Regulated by Pheromonal Signaling in *Caenorhabditis elegans*. Science *329*, 1647–1650.

Yemini, E., Kerr, R.A., and Schafer, W.R. (2011). Tracking Movement Behavior of Multiple Worms on Food. Cold Spring Harb. Protoc. *2011*, pdb.prot067025.

Yu, Z., Chen, X., Zhang, J., Wang, R., and Yin, D. (2013). Transgenerational effects of heavy metals on L3 larva of *Caenorhabditis elegans* with greater behavior and growth inhibitions in the progeny. Ecotoxicol. Environ. Saf. *88*, 178–184.

Zhao, B., Khare, P., Feldman, L., and Dent, J.A. (2003). Reversal Frequency in *Caenorhabditis elegans* Represents an Integrated Response to the State of the Animal and Its Environment. J. Neurosci. *23*, 5319–5328.

Zuur, A.F., Ieno, E.N., and Elphick, C.S. (2010). A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. *1*, 3–14.

Chapter 3: Results

Univariate selection surfaces show extensive directional and stabilizing natural selection on behaviour

Univariate quadratic approximations of the selection surface show pervasive selection on behaviour in itself, or what we can call here behaviour *centrality*, measured by the median, both in NaCl 25 mM – the environment in which the ancestral population has lived – and in NaCl 305 mM, the environment the populations have experienced by generation 50 of experimental evolution.

On one hand, linear elasticities of selection are very similar in both environments for many features, namely in forward and reverse lateral bendings, forward velocity, all turning rate features (turning rate, forward, reverse and stationary turning rates), interval between locomotion bouts – all of these with negative elasticities of selection –, as well as forward and reverse bout durations, interval between reverse bouts, forward, reverse and overall locomotion fractions, forward, reverse and total distances travelled, reverse bout frequency and exploration density – all with positive elasticities of selection. Also similar in both environments, but to a lesser extent, are the elasticities of selection of all regressed length and regressed width features, which are all positive. In these features, selection was stronger in NaCl 305 mM than in NaCl 25 mM, given the higher elasticities of selection found in the first environment (Figure 3.1).

Only stationary lateral bending, net distance travelled and exploration rate was under selection exclusively in NaCl 25 mM. On the other hand, there were seven features under selection only in NaCl 305 mM.

Specifically, negative elasticities of selection were detected for overall turning rate, reverse locomotion velocity and distance to nearest average neighbour, whereas positive elasticities of selection were detected for overall and stationary curvature, forward and stationary longitudinal bendings and standardized distance to nearest average neighbour (Figure 3.1).

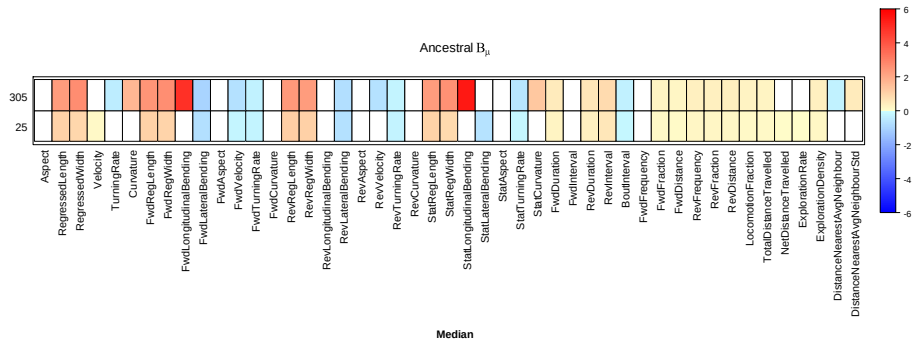


Figure 3.1 – Linear, directional selection on behaviour centrality shown by univariate elasticities of selection pressures on feature median genotypic values. The upper row (305) shows coefficients in NaCl 305 mM and lower row (25) the coefficients in NaCl 25 mM. Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

The univariate quadratic selection coefficients estimated on behaviour medians are also similar in both environments to a high degree (Figure 3.2). There is a markedly negative quadratic selection on all longitudinal bending features in both environments, but also to a lower degree on all regressed width features, curvature during reverse locomotion, forward and reverse bout frequencies, time fraction in reverse locomotion, distance travelled in reverse locomotion and all aspect features (Figure 3.1). There is also widespread quadratic selection that is only specific to NaCl 25 mM and can be seen by the negative elasticities of selection on all

regressed length features, overall, forward and stationary curvatures, forward bout duration and distance to nearest average neighbour. Specific to NaCl 305 mM were the negative quadratic elasticities of selection on time fraction and distance travelled in forward locomotion, overall locomotion fraction, total and net distance travelled, as well as the positive quadratic elasticities of selection on turning rates while on forward and reverse locomotion and also stationary lateral bending.

By looking at the univariate selection surfaces themselves (Appendix 3.1) we can clearly visualize stabilizing selection in all the features with negative quadratic selection coefficients, especially on the longitudinal bending features, on which quadratic selection is very strong. The only exception is reverse lateral bending, in which the optimum value is below any of the genotypic means present in the ancestral population and therefore the quadratic selection detected reveals non-linear directional selection. On the other hand, the positive quadratic selection coefficients detected for stationary lateral bending and both forward and reverse turning rates imply actual cases of disruptive selection (Appendix 3.1). Thus said, selection has indeed favoured intermediate values for most of the features that have a negative quadratic elasticity of selection, whereas it has favoured extreme values in all the features that have a positive quadratic elasticity of selection.

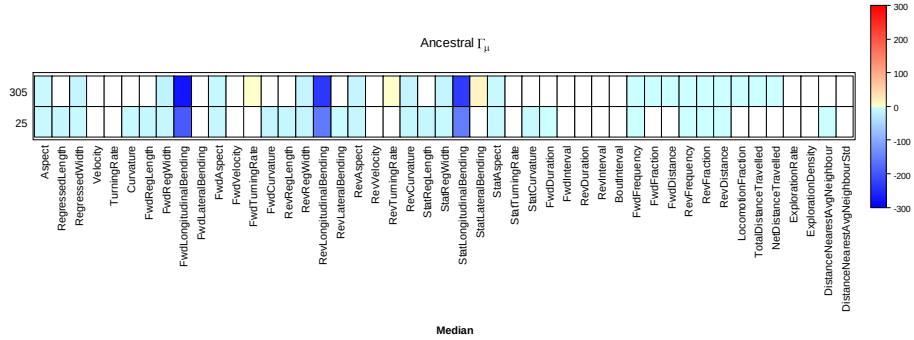


Figure 3.2 – Quadratic selection on behaviour centrality shown by univariate elasticities of selection pressures on feature median genotypic values. The upper row (305) shows coefficients in NaCl 305 mM and lower row (25) the coefficients in NaCl 25 mM. Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

In summary, the univariate selection surfaces in NaCl 25 mM and in NaCl 305 mM are similar for a considerable number of features. It seems that directional selection in both environments has favoured individuals with a higher locomotor activity, which engaged more frequently in locomotion, travelled longer distances, with lower velocity when in forward locomotion and with less curved postures. Even though selection was equally strong for most of these features, in some others selection was stronger in NaCl 305 mM (Figure 3.1). Accompanying directional selection, there was stabilizing selection acting also on postural features and in locomotion patterns, especially on reverse locomotion. This stabilizing selection was exceptionally strong in longitudinal bending features (Figure 3.2).

In NaCl 25 mM specifically, directional selection has favoured less curved positions when individuals were not in locomotion, higher exploration rate and a higher imbalance in the distance travelled, favouring forward

locomotion. Stabilizing selection, in turn, has acted on more postural features – regressed width and curvature features – but also on forward bout duration.

Directional selection occurring specifically in NaCl 305 mM in turn has favoured sharper longitudinal bends during locomotion and more highly curved postures, especially when not in locomotion, but less frequent changes in direction and lower velocity during reverse locomotion; also, directional selection might have favoured less social interaction, thus favouring individuals that generally keep their conspecifics at a higher distance. There was also stabilizing selection in this environment acting on forward and overall locomotor patterns and disruptive selection for turning rate during locomotion and lateral bending when out of locomotion.

Univariate selection surfaces mostly show selection for an increase in behaviour variability

When looking at the univariate directional selection estimates on behaviour variability, measured by the median absolute deviation from the median (MAD), we can observe pervasive directional selection, since most of the features have positive linear elasticities of selection – the cases of all regressed length features, all regressed width features, curvatures overall, in forward and reverse locomotion. In other words, most features here are under selection for an increase in behaviour variability in both environments. The exceptions to this pattern are forward and stationary turning rate and stationary lateral bending, where elasticities of selection are negative, thus there is selection for a decrease in variability of these features (Figure 3.3).

However, directional selection is stronger and more widespread in NaCl 305 mM than in NaCl 25 mM. Only overall velocity – positive coefficient – and interval between bouts – negative coefficient – have been under selection specifically in NaCl 25 mM; in other words, there is a selection for increase in overall velocity variability and a decrease in variability of interval between bouts operating only in NaCl 25 mM. In contrast, there are seven features under selection for variability only in NaCl 305 mM and all of which for an increase in variability – forward and reverse longitudinal bendings, forward and reverse lateral bendings, forward and reverse aspects and interval between forward bouts. It should also be noted that elasticities of selection in NaCl 305 mM are generally higher in absolute terms than in NaCl 25 mM (Figure 3.3).

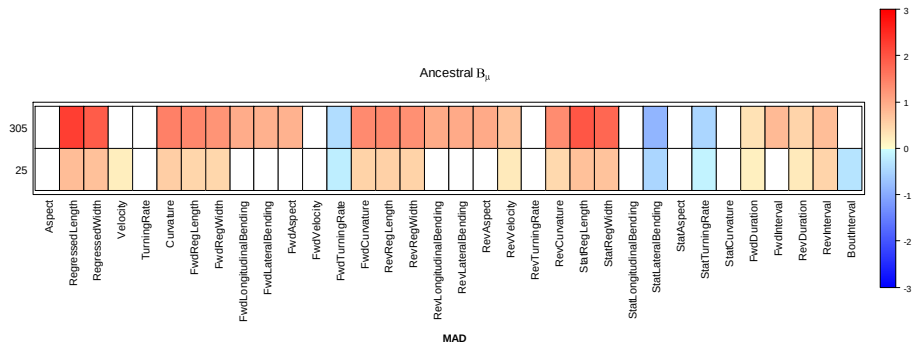


Figure 3.3 – Linear, directional elasticities of selection on behaviour variability genetic values shown by univariate selection pressures on each of the feature MADs. The upper row (305) shows coefficients in NaCl 305 mM and lower row (25) the coefficients in NaCl 25 mM. Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

Quadratic selection on behaviour variability was also extensive, according to these univariate selection surfaces. Most of the coefficients are negative in both environments (Figure 3.4), in the same way as

observed for behaviour median (Figure 3.2). In both environments, negative quadratic elasticities of selection were detected for all aspect features, all velocity features, all longitudinal bending features, reverse bout duration and interval between bouts.

Specifically in NaCl 25 mM, negative quadratic elasticities of selection were detected for variability in overall, forward and stationary regressed lengths, overall regressed width and forward bout duration. In NaCl 305 mM specifically, a positive elasticity of selection was detected for stationary lateral bending and negative elasticities of selection were found for overall and stationary curvature, stationary regressed width and interval between forward locomotion bouts.

The coefficients estimated for MADs also seem to be related to patterns of stabilizing and disruptive selection when we look to the univariate selection surfaces in detail (Appendix 3.2). The exception is reverse longitudinal bending in NaCl 305 mM, in which the fitness optimum is located outside of the domain of the population variation, being thus under non-linear directional selection (Appendix 3.2).

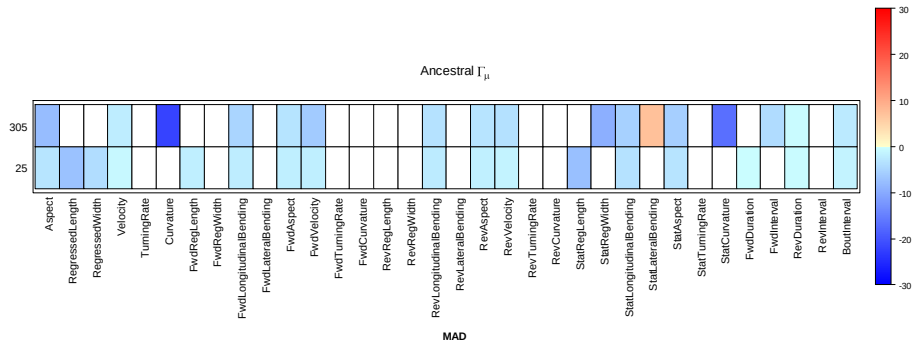


Figure 3.4 – Quadratic selection on behaviour variability shown by univariate elasticities of selection pressures on feature median breeding values. The upper row (305) shows coefficients in NaCl 305 mM and lower row (25) the

coefficients in NaCl 25 mM. Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

In short, widespread directional selection was observed in both environments, yet it was stronger in NaCl 305 mM, favouring mostly an increase in postural variability during and off locomotion. In both of these environments, there was also stabilizing selection on longitudinal bending and velocity features. In NaCl 25 mM only, there was directional selection for a decrease in variability of the interval between forward bouts and stabilizing selection for postural features such as regressed length and width off locomotion and in forward locomotion, but also on forward bout duration. In NaCl 305 mM only, directional selection acted in order to increase variability in bending and aspect features during locomotion; stabilizing selection acted on curvature and regressed width off locomotion and both directional and stabilizing selection have acted on the variability of interval between forward bouts.

No evidence for different strength of directional selection on behaviour variability and centrality

The comparison of the univariate selection coefficients between behaviour medians and MADs would give a good indication of the how important selection on behaviour variability – quantified by a measure of dispersion such as the MAD – when compared to selection on behaviour *centrality* – quantified using a measure of location such as the median. In the previous section, it has been demonstrated that both of these behaviour modes were under extensive selection in environments occurring in the beginning and in the end of the experimental evolution.

Here the question is lies on the relative importance of selection on both these aspects of behaviour, for instance whether behaviour variability is under stronger selection than behaviour centrality or otherwise.

The comparison of the absolute elasticities of selection between behaviour median and MAD shows that there might be a trend for stronger quadratic selection on behaviour median in both environments (Figure 3.5b,d), due to the exceptionally high coefficients regarding curvature features in NaCl 25 mM, on one hand, and regarding longitudinal bending in NaCl 305 mM, on the other (Figure 3.2). In NaCl 25 mM, there is actually statistical significance supporting a stronger quadratic selection on behaviour centrality (Figure 3.5b). However, no statistical evidence was found to reject the possibility that the strength of directional selection is the same in both behaviour modes (Figure 3.5a,c).

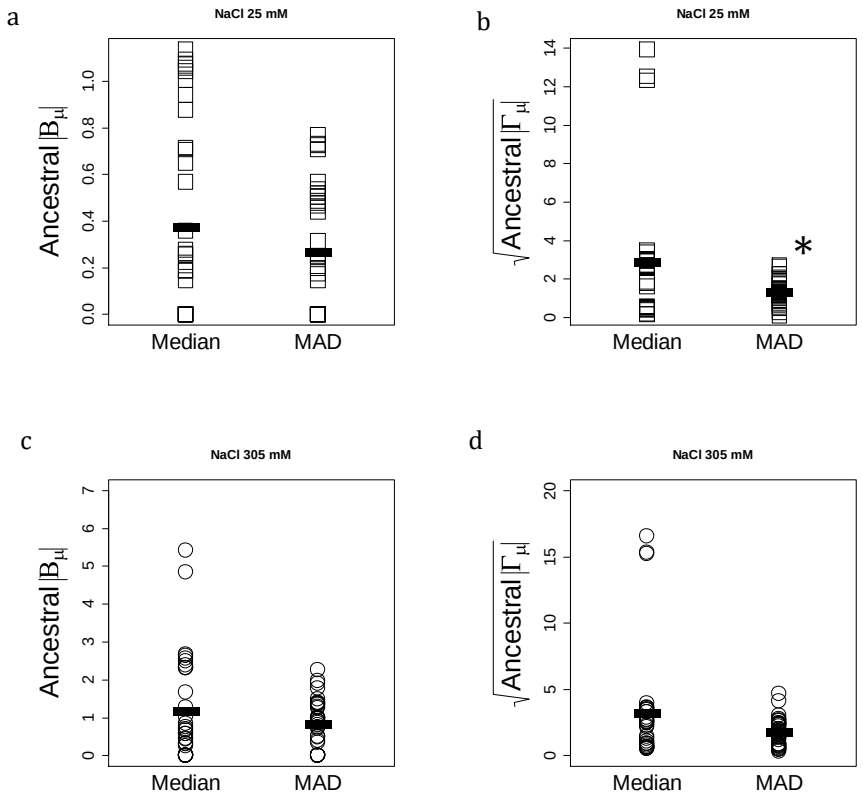


Figure 3.5 – Comparison of elasticities of selection measured in behaviour median and MAD. Each grey point is an absolute value of a selection coefficient shown in figures 3.1 to 3.4 and the black dash is the mean of the values for each group. a. Elasticities of linear selection gradients in NaCl 25 mM: t = 1.2887, df = 56.021, *P* = 0.2028. b. Elasticities of quadratic selection in NaCl 25 mM: t = 2.5373, df = 35.2, *P* = 0.01575(*). c. Elasticities of linear selection gradients in NaCl 305 mM: t = 1.3118, d.f. = 47.188, *P* = 0.1959. d. Elasticities of quadratic selection in NaCl 305 mM: t = 1.9939, d.f. = 37.114, *P* = 0.05355. The quadratic selection coefficients in b and d are square-rooted to ease visualization.

The majority of significant features in the best multivariate selection surfaces of behaviour are behaviour variability features

From the initial 81-feature selection surfaces in each environment, the best linear approximations of the selection surfaces have retained both behaviour variability features and behaviour centrality features. Although the number and identity of these features differs sharply among environments, most of the significant features appearing in the multivariate selection surfaces in both environments are behaviour variability features. Therefore, this scenario would suggest that in both environments there would be more variability features being direcly selected than centrality features. In NaCl 25 mM, the best multivariate selection surface included 36 terms after stepwise regression – 20 behaviour centrality features and 16 behaviour variability features, most of which not having a significant selection coefficient. Regarding behaviour centrality, 12 of the behaviour centrality features – aspect (overall and stationary), regressed length (overall and stationary), regressed width (overall and stationary), velocity (overall), turning rate (forward and reverse), interval between forward locomotion bouts, forward bout frequency and exploration density – and 12 behaviour variability features – variabilities in overall and stationary regressed widths, lateral bending during forward locomotion and off locomotion, velocity and turning rate during forward and reverse locomotion, aspect off locomotion and interval between locomotion bouts had significant selection gradients. Selection favoured a decrease in five features – overall aspect, overall regressed length, regressed width off locomotion, turning rate during reverse locomotion and forward bout frequency – while favouring increases in the remaining seven features – overall regressed width and velocity, regressed length and aspect off locomotion,

interval between forward locomotion bouts, turning rate during forward locomotion and exploration density (Figure 3.6a). In what concerns to behaviour variability, the multivariate selection surface in NaCl 25 mM favoured increases in variability in five features – both velocity and lateral bending during forward locomotion, turning rate during backward locomotion and both regressed width and curvature off locomotion –, while favouring decreases in variability of the remaining seven features – overall regressed width, turning rate during forward locomotion, curvature and velocity during backward locomotion, lateral bending and aspect off locomotion and interval between locomotion bouts (Figure 3.6a). In NaCl 305 mM, the best linear approximation of the multivariate selection surface included 23 terms – 10 centrality features and 13 variability features. Of these, significant selection gradients were estimated for five behaviour centrality features and seven behaviour variability features. The behaviour centrality features under selection were regressed length (overall and forward), forward longitudinal bending, stationary lateral bending, interval between forward bouts and distance travelled in forward locomotion; two of these, overall regressed length and distance travelled in forward locomotion were selected for decrease (for they had negative gradients) and the remaining four – with positive gradients – were selected in order to increase (Figure 3.6b). On the other hand, the behaviour variability features under selection in the multivariate selection surface in NaCl 305 mM were overall velocity, curvature (overall, forward and stationary), reverse and stationary longitudinal bendings and also aspect during reverse locomotion; of these, overall curvature and longitudinal bending during backward locomotion and off locomotion were selected for decrease in variability, whereas the remaining four features were selected for increase in variability (Figure 3.6b). It should be noted here that there are many more centrality and variability features in the multivariate surfaces

under selection for decrease – given the negative selection coefficients (Figure 3.6) –, most of them in opposition to the selection for increase observed in the univariate directional selection coefficients, as seen by the positive directional selection coefficients (Figures 3.1 and 3.3). For instance, in NaCl 25 mM, variability in overall regressed width and velocity during backward locomotion have a positive directional selection coefficient in the respective univariate selection surfaces (Figure 3.3), being therefore selected for increase in variability; in the multivariate selection surface in the same environment, these features have negative directional selection coefficients, suggesting they are actually being directly selected for a decrease in variability (Figure 3.6a). All estimates and associated p-values in multivariate selection surfaces are shown in Appendix 4.1.

In summary, the best multivariate selection surface in NaCl 25 mM has shown selection on postural features during forward locomotion and off locomotion, overall velocity and turning rate during locomotion, while it has also shown selection on the variability of many postural features in every context as well as velocity and turning rate during both forward and backward locomotion and interval between locomotion bouts. In NaCl 305 mM, the best linear approximation of the multivariate selection surface has shown selection on increase in some postural features and decrease in others, selection for larger intervals between for between forward locomotion bouts and smaller distance travelled in forward locomotion. There was also selection for higher variability in overall velocity and some postural features during forward locomotion and off locomotion and selection for smaller variability in overall curvature and longitudinal bending during backward locomotion.

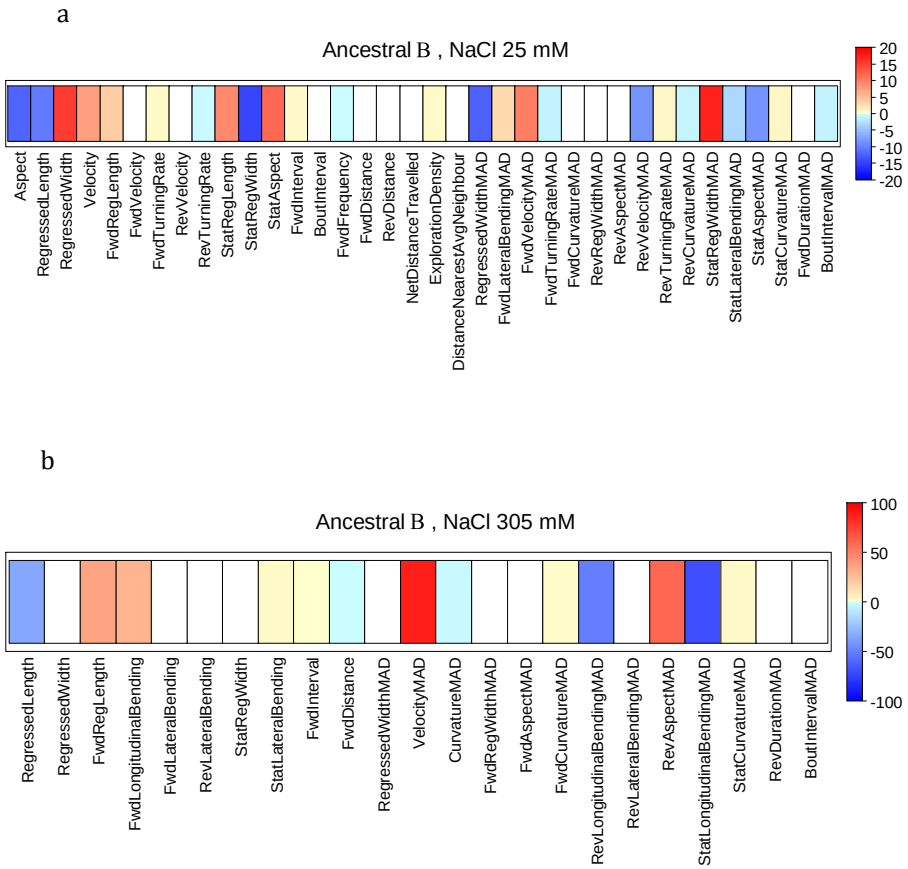


Figure 3.6 – Multivariate selection gradients on the genotypic values of the features included in the best linear approximation of the selection surface in NaCl 25 mM (**a**) and NaCl 305 (**b**). Gradients in NaCl 25 mM (**a**) were transformed to make all significant selection gradients on plot. Positive values were transformed using $B' = \sqrt{B}$ on positive gradients and $B' = -\sqrt{-B}$ on negative gradients. Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

However, given that both 36 – in NaCl 25 mM – and 23 terms – in NaCl 305 mM – are still several terms for regressions and that these regressions included highly redundant terms by definition – *e.g.* regressed length and stationary regressed length in NaCl 25 mM, curvature and forward curvature in NaCl 305 mM –, I have checked whether these approximations included collinear terms, *i.e.* whether the regressions had issues of multicollinearity. The variance inflation factors calculated on each of the features included in both multivariate selection surfaces indeed show very serious multicollinearity issues, with VIF values which were extremely high. In NaCl 25 mM, VIFs up to the order of 10^7 were obtained, which is extremely high; the multicollinearity problem was such that 32 out of the 36 features had a VIF higher than 10 and velocity (centrality) was the only feature with a VIF lower than 3 (Table 3.1). In NaCl 305 mM, the scenario was similarly overwhelming. The maximum VIF observed was much lower, but 20 out of the 23 features had a VIF higher than 10 and all features had a VIF higher than 3 (Table 3.2).

Table 3.1 – Variance inflation factors (VIF) of the features included in the best linear approximation of the selection surface in NaCl 25 mM. Asterisks remark the principal components for which a statistically significant selection gradient was found, which are also the coloured features in Figure 3.6a.

Feature	**VIF**
Aspect	3.19×10^{3} *
RegressedLength	1.15×10^{4} *
RegressedWidth	2.48×10^{3} *
Velocity	1.02*
FwdRegLength	3.52 *
FwdVelocity	97.6
FwdTurningRate	62.7 *
RevVelocity	51.0
RevTurningRate	17.7 *
StatRegLength	9.15×10^{3} *
StatRegWidth	2.57×10^{3} *
StatAspect	3.46×10^{3} *
FwdInterval	17.0 *
BoutInterval	35.1
FwdFrequency	17.1 *
FwdDistance	8.45×10^{7}
RevDistance	3.92×10^{6}
NetDistanceTravelled	6.18×10^{7}
ExplorationDensity	10.2 *
DistanceNearestAvgNeighbour	3.33
RegressedWidthMAD	119 *
FwdLateralBendingMAD	34.9 *
FwdVelocityMAD	21.0 *
FwdTurningRateMAD	39.3 *
FwdCurvatureMAD	42.2
RevRegWidthMAD	89.0
RevAspectMAD	76.5
RevVelocityMAD	33.5 *
RevTurningRateMAD	25.1 *
RevCurvatureMAD	17.2 *
StatRegWidthMAD	137 *
StatLateralBendingMAD	34.8 *
StatAspectMAD	95.3 *
StatCurvatureMAD	13.9 *
FwdDurationMAD	29.0
BoutIntervalMAD	9.65 *

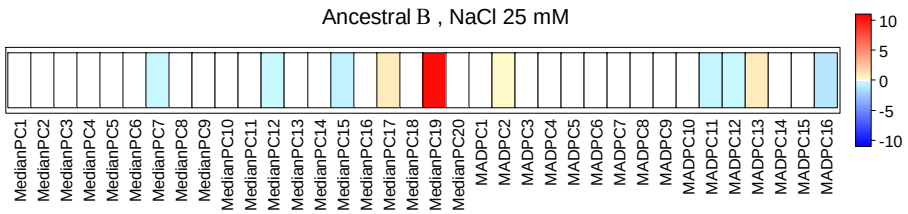
Table 3.2 – Variance inflation factors (VIF) of the features included in the best linear approximation of the selection surface in NaCl 305 mM. Asterisks remark the principal components for which a statistically significant selection gradient was found, which are also the coloured features in Figure 3.6b.

Feature	**VIF**
RegressedLength	174.2141 *
RegressedWidth	690.6953
FwdRegLength	188.7387 *
FwdLongitudinalBending	29.64777 *
FwdLateralBending	53.77694
RevLateralBending	12.49494
StatRegWidth	694.5701
StatLateralBending	39.5607 *
FwdInterval	3.647427 *
FwdDistance	11.77392 *
RegressedWidthMAD	19.7188
VelocityMAD	7.960777 *
CurvatureMAD	48.33981 *
FwdRegWidthMAD	55.85208
FwdAspectMAD	49.12389
FwdCurvatureMAD	34.91026 *
RevLongitudinalBendingMAD	10.8829 *
RevLateralBendingMAD	7.100961
RevAspectMAD	16.5825 *
StatLongitudinalBendingMAD	23.43998 *
StatCurvatureMAD	37.80747 *
RevDurationMAD	13.79285
BoutIntervalMAD	3.574801

Multivariate selection surface on principal components shows as well selection on behaviour median and MAD principal components

Starting from the genotypic values of the features included in the linear approximations of the selection surface above, I have performed, for each environment, two principal components analyses, one on the behaviour centrality features and another principal components analysis on the variability features. Then, I have performed a regression using all principal components – 36 principal components in NaCl 25 mM, 23 in NaCl 305 mM. In NaCl 25 mM, we can observe selection in five of the median principal components – median PCs 7, 12, 15, 17 and 19 – and five of the MAD principal components – MAD PCs 2, 11, 12, 13 and 16 (Figure 3.7a). In NaCl 305 mM, we observed selection on five of the median principal components – median PC1-PC4 and median PC9 – and five of the MAD principal components – MADPCs 1, 2, 6, 7 and 13 (Figure 3.7b).

a



b

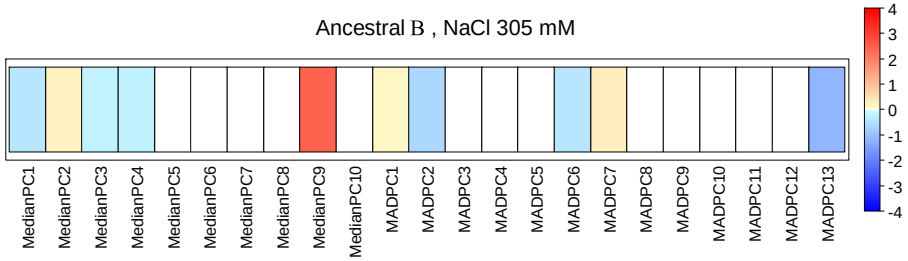


Figure 3.7 – Selection gradients estimated on the principal components of behaviour – stated as MedianPCs – and behaviour variability – stated as MADPCs – in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

To check for existence of multicollinearity in these regressions using the principal components, I have also calculated the variance inflation factors of the principal components. The extent of multicollinearity was not as high as the observed for the multivariate surfaces with the original features, yet there still was serious multicollinearity associated to some of the principal component features. In NaCl 25 mM, seven out of the 36

features had VIF higher than 10 and 14 of the features had VIFs higher than 3 (Table 3.3). In NaCl 305 mM, four of the 23 features had VIFs higher than 10 and 11 of them had VIFs above 3 (Table 3.4).

Table 3.3 – Variance inflation factors (VIF) of feature median and MAD principal components included in a linear approximation of the selection surface in NaCl 25 mM using principal components. Asterisks remark the principal components for which a statistically significant selection gradient was found.

Feature	VIF
MedianPC1	49.704934
MedianPC2	40.834939
MedianPC3	21.434094
MedianPC4	9.671297
MedianPC5	2.878289
MedianPC6	3.601906
MedianPC7*	4.249887
MedianPC8	2.769667
MedianPC9	4.292773
MedianPC10	1.79838
MedianPC11	2.354381
MedianPC12*	1.499666
MedianPC13	2.284275
MedianPC14	2.457695
MedianPC15*	1.872893
MedianPC16	1.555853
MedianPC17*	1.559895
MedianPC18	1.623942
MedianPC19*	1.392136
MedianPC20	1.188944

Feature	VIF
MADPC1	32.145244
MADPC2*	37.284871
MADPC3	25.021441
MADPC4	26.256364
MADPC5	2.749104
MADPC6	4.4866
MADPC7	5.833673
MADPC8	2.75201
MADPC9	4.701027
MADPC10	2.905292
MADPC11*	2.568031
MADPC12*	1.495977
MADPC13*	2.684259
MADPC14	1.421763
MADPC15	1.549998
MADPC16*	1.605679

Table 3.4 – Variance inflation factors (VIF) of feature median and MAD principal components included in a second linear approximation of the selection surface in NaCl 305 mM, this one using principal components. Asterisks remark the principal components for which a statistically significant selection gradient was found.

Feature	VIF
MedianPC1*	27.872131
MedianPC2*	7.430421
MedianPC3*	8.703081
MedianPC4*	5.900407
MedianPC5	3.298008
MedianPC6	1.441834
MedianPC7	1.360914
MedianPC8	1.229636
MedianPC9*	1.920093
MedianPC10	1.19395

Feature	VIF
MADPC1*	10.679787
MADPC2*	20.001015
MADPC3	3.290509
MADPC4	3.884577
MADPC5	11.359083
MADPC6*	3.416589
MADPC7*	2.109239
MADPC8	2.300815
MADPC9	1.366418
MADPC10	1.519273
MADPC11	1.123603
MADPC12	1.372669
MADPC13*	1.351067

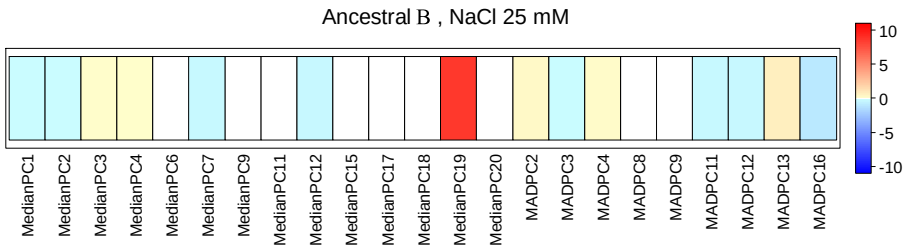
To face once again the multicollinearity issue, this time on the principal components, I have performed a stepwise regression taking this principal components model as a starting model.

In NaCl 25 mM, the best linear approximation of the multivariate selection surface using principal components has retained 23 of the 36 initial features and selection was detected on 14 principal components, more than the 10 components in which selection was detected in the model with all the principal components. The principal components with statistically significant selection gradients in the full principal components model were all retained in this best linear approximation, but two of them have lost statistical significance – Median PCs 15 and 17 – because the absolute values of the respective coefficients were sharply lower. From the remaining seven features, MAD PC12 remained with an

identical selection gradient estimate, three principal components had a similar estimate – median PC7, median PC12 and MAD PC2 – and the other four – Median PC19 and MAD PCs 11, 13 and 16 – have remained statistically significant, despite having considerably lower estimates in absolute terms (Appendix 4.4). Thus said, of the 14 components in which statistically significant selection gradients were found, eight principal components were statistically significant in both selection surfaces and the remaining six components – median PCs 1 to 4 and MAD PCs 3 and 4 – have gained statistical significance due to the lower standard errors of the estimates obtained; in three of these six components – median PCs 3 and 4, MAD PC4 –, the estimates increased considerably when compared to the ones obtained in the full model (Appendix 4.4).

In NaCl 305 mM, in turn, the best linear approximation of the selection surface retained 14 of the 23 principal components of the full model and selection was detected on less principal components. All of the 10 principal components showing statistically significant selection coefficients in the full principal components model were retained in the best model, yet only 8 of them remained statistically significant. Two of these features had very similar estimates in the two models – MedianPC2 and MADPC7 – and the other six had lower estimates in absolute value in the best model but remained statistically significant – MedianPC1, Median PC3, MedianPC9, MADPC2, MADPC6 and MADPC13. The remaining two selection coefficients of principal component have decreased considerably in absolute value and lost statistical significance – MedianPC4 and MADPC1. In contrast, the selection coefficient of MedianPC10 has increased in absolute value in the best model and has gained statistical significance (Figure 3.8b; Appendix 4.4).

a



b

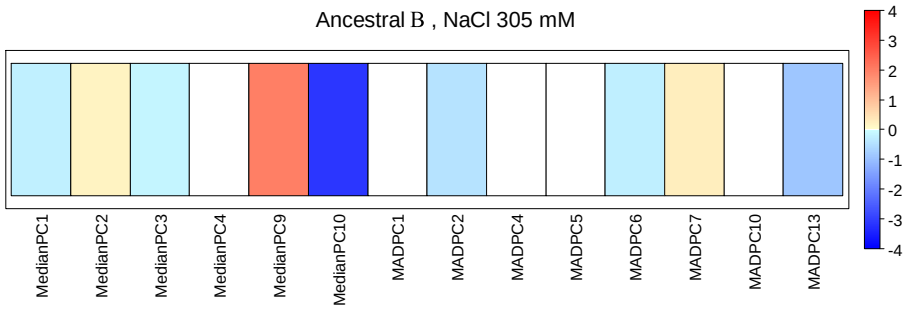


Figure 3.8 – Selection gradients estimated on the best linear approximations of the selection surfaces using principal components of behaviour centrality – stated as MedianPCs – and of behaviour variability – stated as MADPCs – both in NaCl 25 mM (**a**) and in NaCl 305 mM (**b**). Blank rectangles show features in which there was no statistical evidence to reject the null hypothesis of no selection.

In summary, selection coefficient estimates were unstable in the context of a multivariate selection surface in both environments and dependent on the features included in the models to the point of changing significance of selection on some principal components. This estimate instability is very likely the consequence of the high degree of multicollinearity still observed in the full principal components models (Tables 3.3 and 3.4). Nevertheless, selection could still be robustly

detected in a given direction on the majority of the principal components – eight out of the previous 14 principal components in NaCl 25 mM and eight out of the previous 10 principal components in NaCl 305 mM.

I have also calculated the variance inflation factors associated to the principal components preserved in these best linear approximations to check for multicollinearity in these surfaces and still found some features with very high VIF, which shows that these surfaces, despite being a result of simpler models, are still plagued by a high degree of multicollinearity. In NaCl 25 mM, seven of the principal components had a VIF higher than 3, two of which higher than 10 (Table 3.5). In NaCl 305 mM, eight of the principal components had VIFs higher than 3, two of which were higher than 10 (Table 3.6).

Table 3.5 – Variance inflation factors (VIF) of feature median and MAD principal components included in the best linear approximation of the selection surface in NaCl 25 mM using principal components. Asterisks remark the principal components for which a statistically significant selection gradient was found.

Feature	VIF
MedianPC1*	13.0264
MedianPC2*	8.378587
MedianPC3*	6.042104
MedianPC4*	3.15753
MedianPC6	1.752158
MedianPC7*	1.467464
MedianPC9	1.946705
MedianPC11	1.737587
MedianPC12*	1.166991
MedianPC15	1.349684
MedianPC17	1.187929
MedianPC18	1.274631
MedianPC19*	1.158045
MedianPC20	1.086432

Feature	VIF
MADPC2*	13.95825
MADPC3*	7.351508
MADPC4*	8.830464
MADPC8	1.781628
MADPC9	2.525051
MADPC11*	1.432386
MADPC12*	1.200359
MADPC13*	1.594191
MADPC16*	1.323164

Table 3.6 – Variance inflation factors (VIF) of feature median and MAD principal components included in the best linear approximation of the selection surface in NaCl 305 mM using principal components. Asterisks remark the principal components for which a statistically significant selection gradient was found.

Feature	VIF
MedianPC1*	16.3092
MedianPC2*	5.691585
MedianPC3*	4.886783
MedianPC4	3.001668
MedianPC9*	1.544926
MedianPC10*	1.133149

Feature	VIF
MADPC1	7.642927
MADPC2*	11.2162
MADPC4	3.117664
MADPC5	6.660039
MADPC6*	2.153627
MADPC7*	1.475618
MADPC10	1.354526
MADPC13*	1.15222

Evolutionary response of behaviour centrality is widespread and substantially different in the populations and inbred lines

In the populations, we can observe little evolution of behaviour centrality (median) features, with more features evolving in NaCl 305 mM than in NaCl 25 mM (Figure 3.9a). In the first environment, there was evolution of increased stationary longitudinal bending, stationary lateral bending and standardized distance to nearest neighbour. Thus said, individuals tended to be more sharply bent when not locomoting and also keep higher distances from their conspecifics. In the second environment, there was only evolution of regressed width during forward locomotion, which also points to a specific postural evolution towards a more sharply bent posture, but during forward locomotion.

In the inbred lines, the evolutionary response observed is very different (Figure 3.9b). In both environments, there also was evolution of

increased regressed width during forward locomotion – as observed in the populations in NaCl 25 mM –, higher interval between reverse locomotion bouts, higher exploration density, lower distance to nearest average neighbour and higher standardized distance to nearest average neighbour. In NaCl 25 mM specifically, there was evolution of all regressed length features, interval between forward locomotion bouts and duration of forward locomotion bouts. In NaCl 305 mM specifically, there was an increase in all aspect, regressed width features, longitudinal bending during forward and reverse locomotion, as well as reverse bout duration, time fraction and frequency. In contrast, there was also evolution of lower velocity and turning rate during forward locomotion. In summary, there was evolution of different postural features in the two environments, with more features evolving in NaCl 305 mM; there was also evolution of higher distances between individuals, exploratory density and locomotor activity, this one more focused on forward locomotion in NaCl 25 mM and on reverse locomotion in NaCl 305 mM.

This sharp difference in the evolutionary responses of populations and inbred lines from them derived could be due to different statistical power associated to hypotheses testing in inbred line and population datasets. One can see whether that could be possible by plotting the evolutionary responses of inbred lines in one axis and of populations in the other. In such a case, we would observe population and inbred line responses falling into the odd quadrants of such a plot. This observation would reveal that populations and inbred lines have evolutionary responses in the same direction (*i.e.* both would either decrease or increase). The comparison between population and inbred line evolutionary responses in the median shows that most of the features actually show those responses in the same direction both in populations and inbred lines, for most of the features actually fall in the odd quadrants of these plots (Figure 3.10). There are, however, many features in which the trends are

opposite in populations and inbred lines, more in NaCl 25 mM (Figure 3.10a) than in NaCl 305 mM (Figure 3.10b). In the case of NaCl 25 mM (Figure 3.10a), there are four features in the second quadrant (negative inbred response and positive population response) and 13 features in the fourth quadrant (positive inbred response and negative population response). Of these 17 features, one can only say regressed length during reverse locomotion and exploration density have actually evolved only in the inbred lines and the populations follow the opposite trend. Although the remainder also show opposite evolutionary dynamic trends in populations and inbred lines, I do not have evidence to say they have evolved differentially in populations and inbred lines because evolutionary response was not detected in either of them. In the case of NaCl 305 mM, four features were also found in the second quadrant and seven in the fourth quadrant; of these, only velocity and turning rate during forward locomotion (second quadrant), as well as interval between reverse locomotion bouts and regressed width during forward locomotion (fourth quadrant), in which there is an evolutionary response found in the inbred lines and not in the populations. In the remainder there was also no response found in either populations or inbred lines.

All in all, the differences in statistical power of population and inbred line datasets may indeed justify the difference in evolutionary responses observed in those, especially in those features lying in the odd quadrants. The evolution only in the inbred lines of features lying both in the second and fourth quadrants also points to the possibility that there is a differential statistical power in the inbred line and population datasets; yet, it also brings the possibility of a biological effect of inbreeding in the expression and evolution of behaviour.

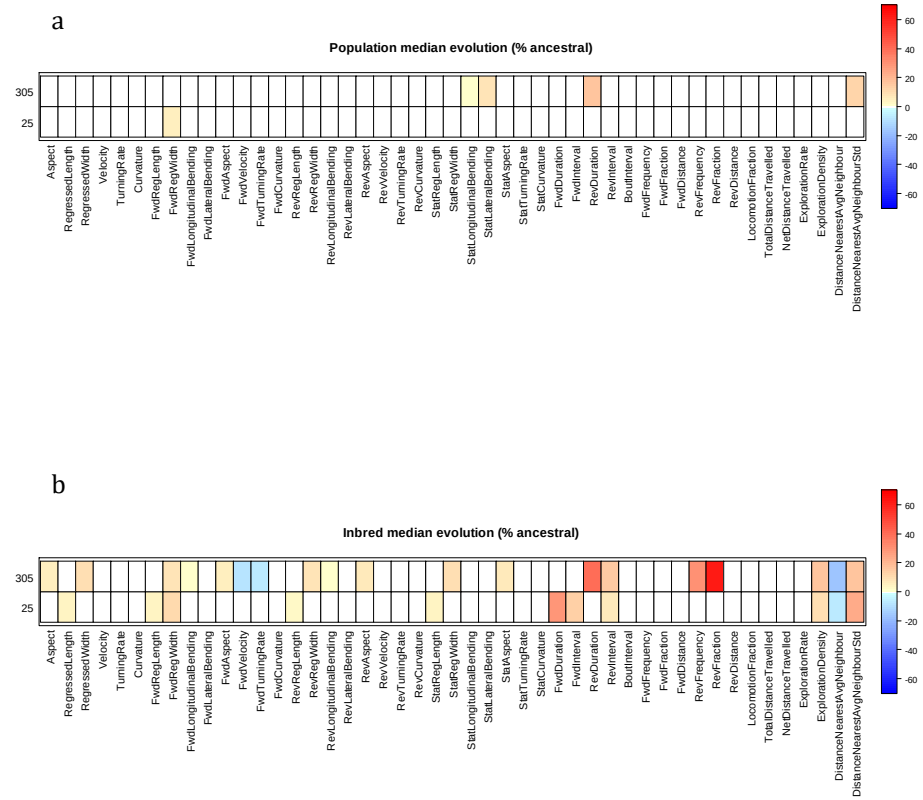


Figure 3.9 – Evolution of behaviour centrality, measured by the median. The blank squares show features in which there was no statistically significant evolutionary response. The upper row (305) shows evolutionary responses in NaCl 305 mM and lower row (25) the coefficients in NaCl 25 mM. Evolutionary responses are measured as a percentage of the respective ancestral values. **a.** Evolution observed in the populations. **b.** Evolution observed in the inbred lines derived from the populations.

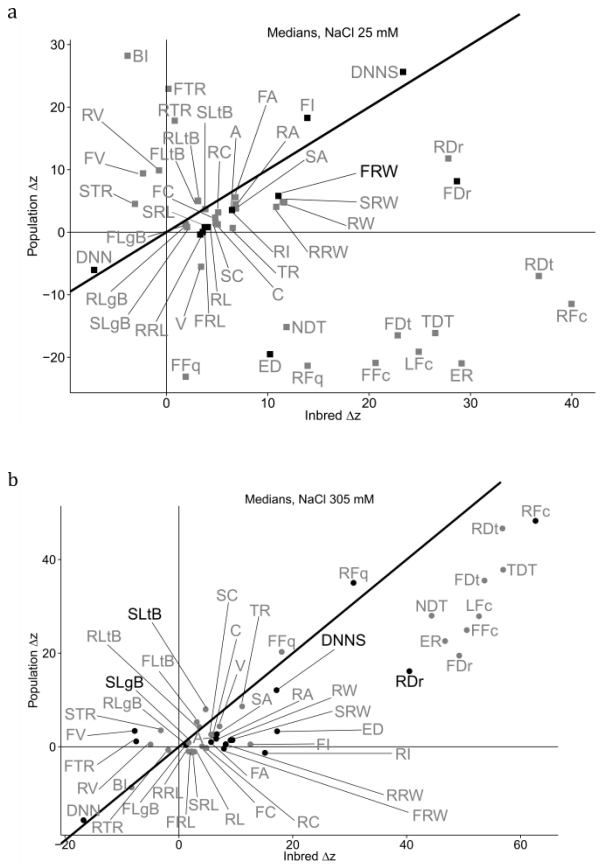


Figure 3.10 – Biplots of evolutionary responses of behaviour centrality (median) in inbred lines and populations, both in NaCl 25 mM (**a**) and in NaCl 305 mM (**b**). Evolutionary responses are measured as a percentage of the respective ancestral values. The diagonal is the bisectrix that that unites the points in which the evolutionary responses are equal in both populations and inbred lines.

Behaviour variability has mostly increased upon evolution in both populations and inbred lines

Regarding behaviour variability, evolutionary response in the populations was drastically different according to the environment, being far more widespread in NaCl 305 mM than in NaCl 25 mM. In this last

environment, only increases in variability of overall and stationary regressed width, as well as a decrease of variability in the interval between reverse bouts have been observed (Figure 3.11a). In NaCl 305 mM, traits mostly related to body posture both during forward and reverse locomotion – the case of regressed width, lateral bending and aspect – and both during reverse locomotion and off locomotion – such as the case of longitudinal bending – have increased in variability. Turning rate during forward locomotion has increased in variability as well (Figure 3.11a).

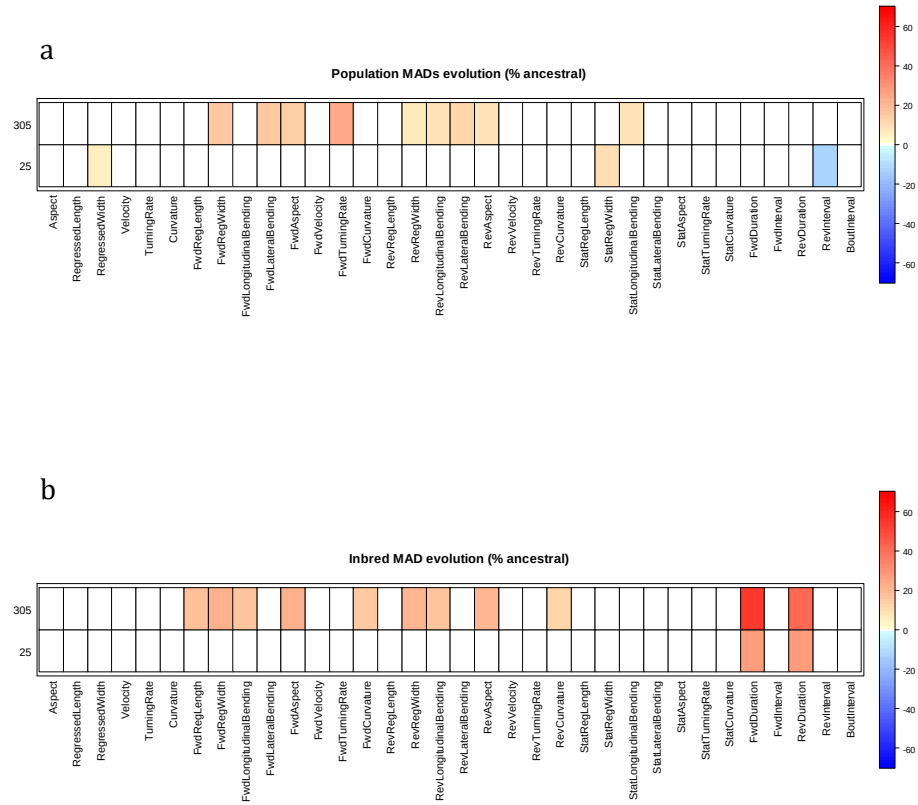


Figure 3.11 – Evolution of behaviour variability, measured by the MAD. The blank squares show features in which there was no statistically significant

evolutionary response. **a.** Evolution observed in the populations. **b.** Evolution observed in the inbred lines derived from the populations.

The evolution observed in the inbred lines has some similarities, but also some differences relative to the observed in the populations. First of all, there was an increase in variability in duration of both forward and reverse locomotion bouts, in both environments (Figure 3.11b). In NaCl 25 mM specifically, no other traits have evolved in terms of variability besides the aforementioned ones. In NaCl 305 mM, variability has also increased in traits related to body posture, as observed in populations; namely, variability has also increased in aspect and regressed width during forward and reverse locomotion. Yet, there was neither evolution of lateral bendings, nor of turning rate, nor of longitudinal bending off locomotion; instead, variability has increased in both longitudinal bending and curvature during forward and reverse locomotion (Figure 3.11b).

It is also possible that the differences between evolutionary responses of behaviour variability in the populations and in the inbred lines are also due to differential statistical power in inbred line and population datasets. By plotting in one axis the evolutionary responses of inbred lines and in another axis the evolutionary responses in populations, one can observe that the majority of the features lie in the first quadrant in both environments (Figure 3.12). Nevertheless, lying in the even quadrants were nine features in NaCl 25 mM (Figure 3.12a) and two features in NaCl 305 mM (Figure 3.12b). Of these traits, only forward bout duration and interval between reverse bouts, both in NaCl 25 mM, have actually evolved, either only in the inbred lines or only in the populations, respectively (Figure 3.12a). In summary, for most of the features, behaviour variability has the same trend in populations and

inbred lines and differential evolution in these might be due to differential statistical power; in contrast, there are only these two aforementioned features for which we can say that there is likely a biological effect of inbreeding in their expression and, possibly, in their evolution.

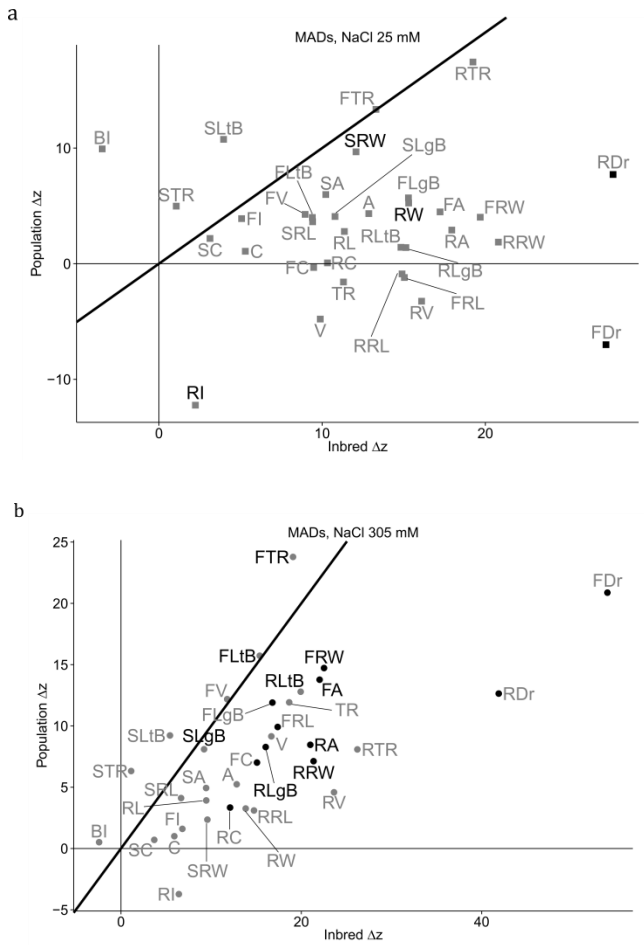


Figure 3.12 – Biplots of evolutionary responses of behaviour variability (MAD) in inbred lines and populations, both in NaCl 25 mM (**a**) and in NaCl 305 mM (**b**). Evolutionary responses are measured as a percentage of the respective ancestral values. The diagonal is the bisectrix that that unites the points in which the evolutionary responses are equal in both populations and inbred lines.

Evolutionary responses in behaviour centrality and variability are more congruent with the univariate rather than the multivariate selection coefficients

In order to have a glimpse on how well could the aforementioned selection surfaces fit the actual evolutionary responses observed in what I have been calling as behaviour centrality, one can plot the directional selection gradients of these selection surfaces in one axis and the evolutionary responses in the other axis and see in which quadrant these points lie. The rationale applied to these plots is the same as the one applied in the previous figure: the features lying in the odd quadrants are the ones in which directional selection and evolutionary response occurred in the same direction and the features lying in the even quadrants are the ones in which directional selection and evolution occurred in opposite directions.

In NaCl 25 mM, the plot of univariate directional selection gradients and evolutionary responses shows that the only feature for which both significant selection gradient and evolutionary response in populations were detected – regressed width while in forward locomotion – has actually increased with evolution and this evolution was in the same direction of selection, for the selection gradient estimated was positive, therefore selection does favour this increase (Figure 3.13a). In NaCl 305 mM, in turn, all three features for which both selection and evolutionary response in populations were detected – reverse bout duration, standardized distance to nearest neighbour and stationary longitudinal bending – have actually evolved in the same direction of selection as well (Figure 3.13b). The majority of features in both environments, however, were under selection but did not evolve (Figure 3.13). The exception goes

to stationary lateral bending, which has evolved although directional selection was not detected for it (Figure 3.13b).

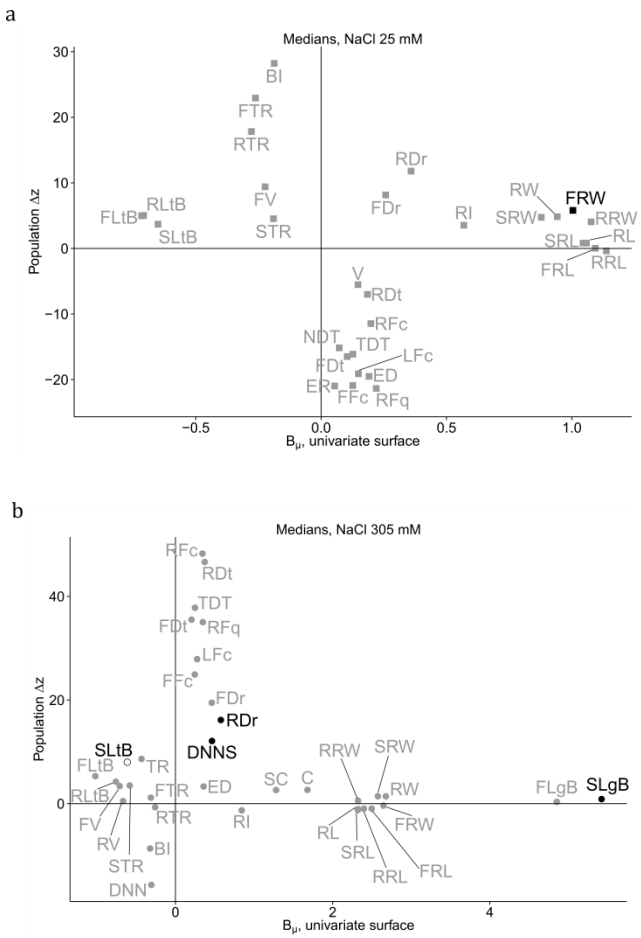


Figure 3.13 – Biplots of the univariate directional elasticities of selection on behaviour centrality (*x*-axis) and the respective evolutionary responses observed in the populations (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. The features for which neither directional selection nor evolution were detected are not represented in the graph.

When the evolution in the inbred lines is plotted instead of population evolution, the congruence between directional selection and evolution is more striking, for more behaviour centrality features have evolved in the inbred lines and all of these features have evolved in the direction pointed by the respective selection coefficients in both environments (Figure 3.14). Yet, not only most of the features were under selection and did not evolve, but also there were features that evolved without concomitant selection detected, such as interval between forward locomotion bouts, distance to nearest neighbour and also its standardized counterpart – in NaCl 25 mM (Figure 3.14a) – and all aspect features – in NaCl 305 mM (Figure 3.14b).

The comparison between evolutionary responses of behaviour variability in the populations and the univariate linear elasticities of selection shows that most of the features have actually evolved in the direction favoured by univariate selection. In NaCl 25 mM, evolutionary response matched the univariate selection surfaces in two features, overall and stationary regressed width; the other feature that evolved – interval between reverse bouts – has evolved in the opposite direction to the one suggested by univariate selection (Figure 3.15a). In NaCl 305 mM, seven of the nine features that evolved – lateral bendings during forward and reverse locomotion, aspect during forward and reverse locomotion, regressed width during forward and reverse locomotion and longitudinal bending during reverse locomotion – did so in the direction favoured by selection (Figure 3.15b); one of the features has evolved despite undetectable selection – longitudinal bending off locomotion – and another feature – turning rate during forward locomotion – has evolved in the opposite direction to the one favoured by selection (Figure 3.15b).

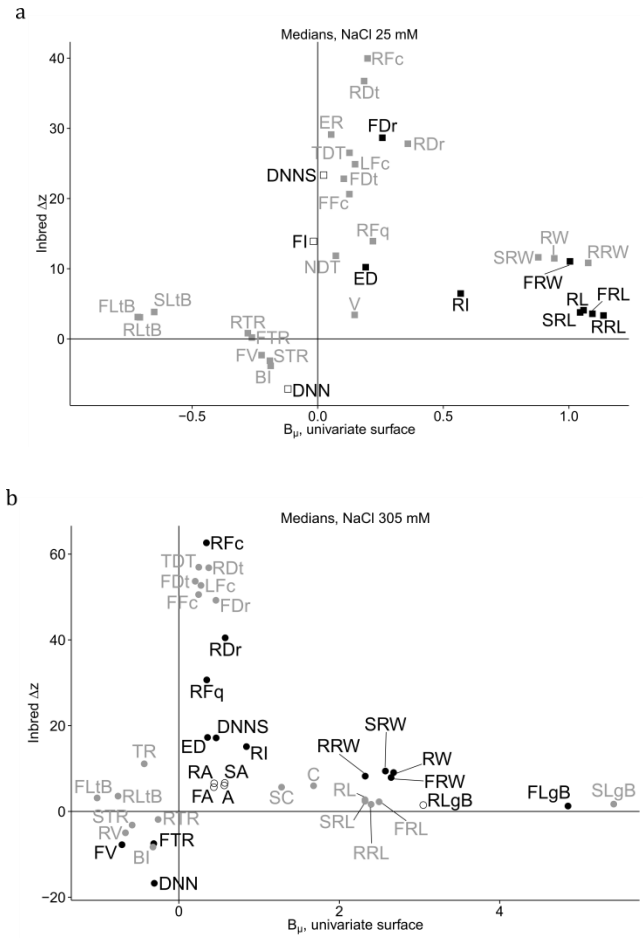


Figure 3.14 – Biplots of the univariate directional elasticities of selection on behaviour centrality (*x*-axis) and the respective evolutionary responses observed in the inbred lines (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. The features for which neither directional selection nor evolution were detected are not represented in the graph.

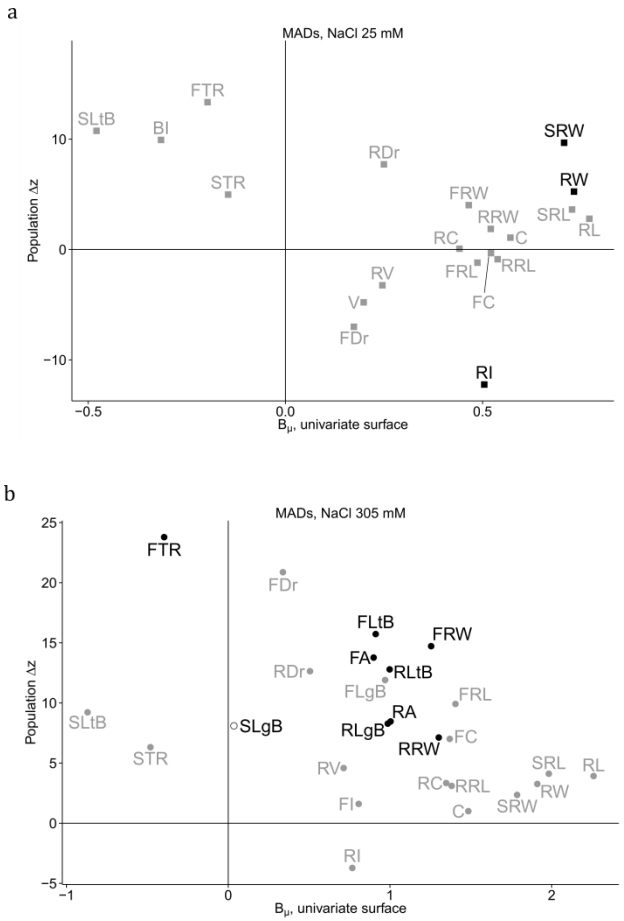


Figure 3.15 – Biplots of the univariate directional elasticities of selection on behaviour variability (*x*-axis) and the respective evolutionary responses observed in the populations (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. The features for which neither directional selection nor evolution were detected are not represented in the graph.

The same kind of comparison, this time with the evolutionary responses of behaviour variability in the inbred lines, shows an even more striking congruence between selection and evolution to the one found in populations. In both environments, all the features that evolved lie in the first quadrant of the respective graphs (Figure 3.16), hence all evolved in the direction favoured by selection.

These directional selection *versus* evolution plots were also made to assess how congruent the multivariate selection surfaces were with the evolutionary responses observed. They would also allow a qualitative comparison between univariate and multivariate selection coefficients in how well they may predict evolutionary responses. The comparison between selection gradients of the best linear approximation of the multivariate selection surfaces using the original features and the evolutionary responses in the populations in both environments shows that roughly half of the features that have evolved in the populations had multivariate selection coefficients pointing to the same direction, whereas the other half had selection coefficients pointing to the opposite direction (Figure 3.17). To be more specific, in NaCl 25 mM, regressed variability in stationary regressed width has evolved in the same direction suggested by the respective multivariate selection coefficient, whereas regressed width overall has evolved in the opposite direction (Figure 3.17a); in NaCl 305 mM, three features – stationary lateral bending and variability in aspect during reverse locomotion and regressed width during forward locomotion – have evolved in the direction suggested by selection, whereas two other features – stationary longitudinal bending and variability in longitudinal bending during reverse locomotion – have evolved in the opposite direction to the suggested by selection (Figure 3.17b). There were also, in NaCl 305 mM, two features that evolved without detectable selection (Figure 3.17b).

There is also, in both environments, a very high number of features which were under selection but did not actually evolve (Figure 3.17).

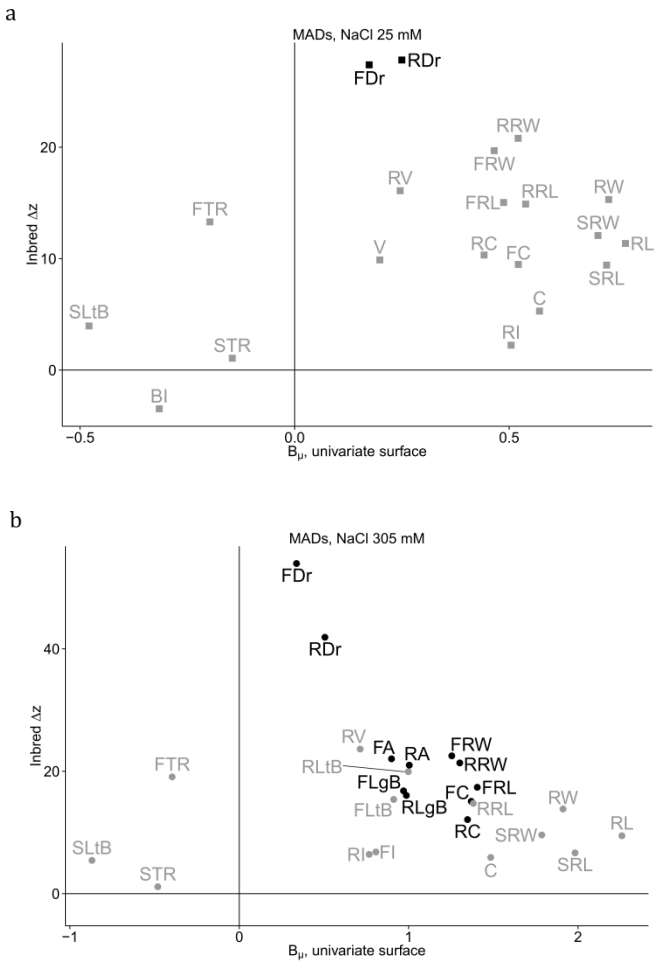


Figure 3.16 – Biplots of the univariate directional elasticities of selection on behaviour variability (*x*-axis) and the respective evolutionary responses observed in the inbred lines (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey

letters). Evolutionary responses are measured as a percentage of the respective ancestral values. The features for which neither directional selection nor evolution were detected are not represented in the graph.

The comparison between coefficients from the linear approximation of the multivariate selection surface and the evolutionary responses found in the inbred lines reveals also that some of the features do evolve in the direction suggested by these multivariate selection coefficients. In NaCl 25 mM, seven features that are part of the multivariate selection surface do evolve and four of these – interval between forward locomotion bouts, exploration density, regressed lengths during forward locomotion and off locomotion – have evolved in the direction suggested by selection; two features – distance to nearest neighbour and variability in forward bout duration – have actually evolved without a detectable selection coefficient and one feature – overall regressed length – has evolved in the opposite direction to the one suggested by selection (Figure 3.18a). In NaCl 305 mM, nine features that were part of the multivariate selection surface have actually evolved, three of which – forward longitudinal bending, variability in reverse bout duration and in curvature during forward locomotion – in the direction suggested by selection; five of the features – overall, forward and stationary regressed width, variability in reverse bout duration and variability in aspect during forward locomotion – have evolved despite undetectable selection and one feature – variability in longitudinal bending during reverse locomotion – has evolved in the direction opposite to the one pointed by selection (Figure 3.18b). The majority of features in the multivariate selection surfaces in both environments were under selection but did not evolve (Figure 3.18).

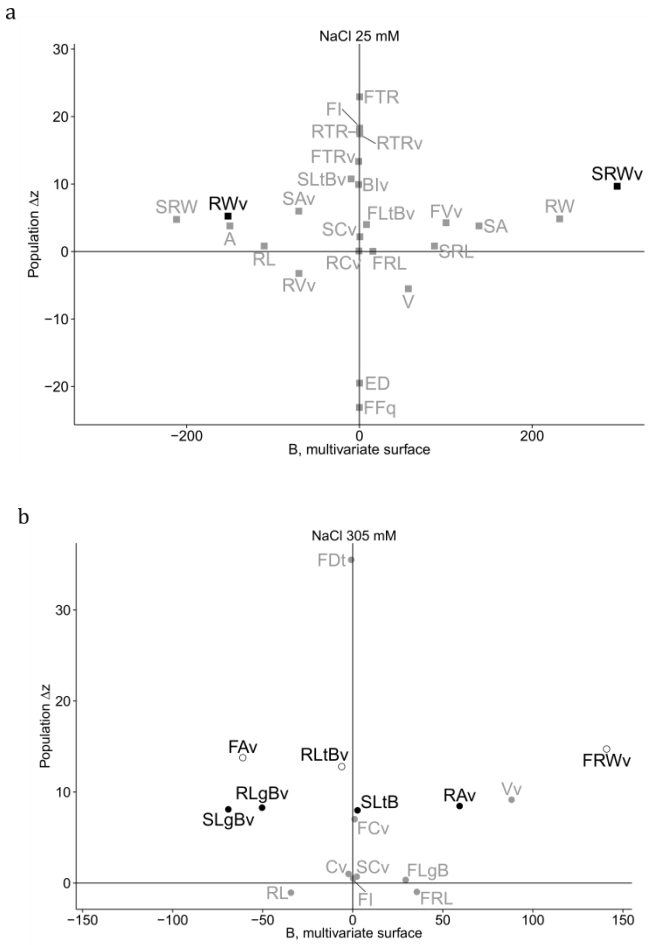


Figure 3.17 – Biplots of the multivariate directional selection gradients on behaviour (*x*-axis) and the respective evolutionary responses observed in the populations (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). Only features present in the best multivariate selection surface for each environment are present in the respective graphs. The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. Features labelled with the suffix *v* are variability features, as opposed to centrality features, without suffix. The features present the multivariate selection surfaces for which neither directional selection nor evolution were detected were not plotted.

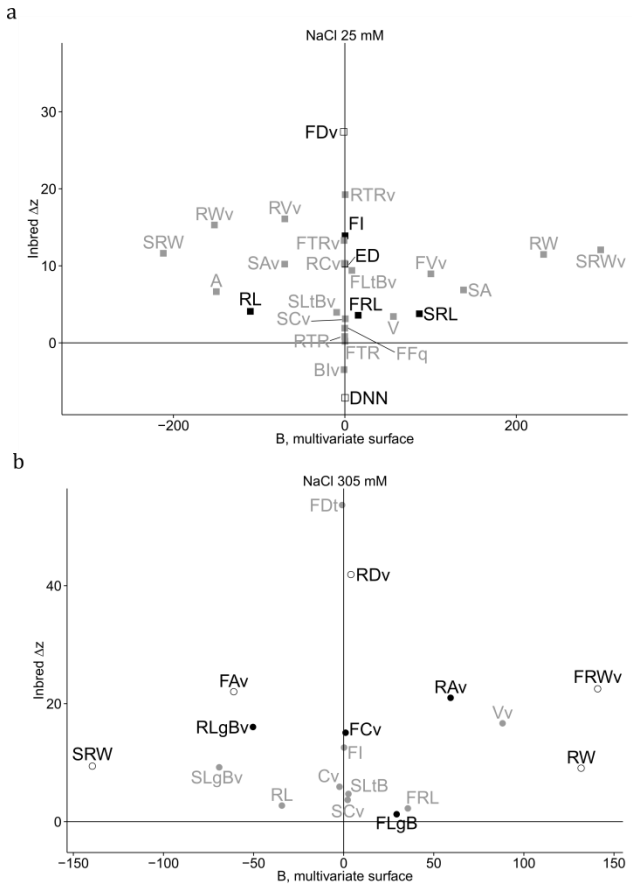


Figure 3.18 – Biplots of the multivariate directional elasticities of selection on behaviour (*x*-axis) and the respective evolutionary responses observed in the inbred lines (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). Only features present in the best multivariate selection surface for each environment are present in the respective graphs. The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. Features labelled with the suffix *v* are variability features, as opposed to centrality features, which carry no suffix. The features present the multivariate selection surfaces for which neither directional selection nor evolution were detected are not shown.

In summary, more traits seem to evolve according to the univariate directional selection coefficients than according to the selection gradients taken from the multivariate selection surfaces. This is not surprising due to the multicollinearity observed in the multivariate selection surfaces. The same sort of comparison was made, this time using the best multivariate selection surfaces using principal components of the features present in the aforementioned surfaces using the original features. Because multicollinearity in the multivariate selection surfaces was less severe than the one with the original features, one could expect that the multivariate surface using principal components would be more congruent with the actual evolutionary response than the surfaces with the original features.

The comparison between these multivariate selection surfaces using principal components and evolutionary responses in the populations do not show that expected improvement in congruence between selection and evolution. In NaCl 25 mM, two principal components have evolved and were also under selection, one of which – MAD PC12 – has evolved according to suggested by the selection surface and the other one – MAD PC11 – has evolved in the opposite direction. There was also one principal component – median PC15 – that has evolved under undetectable selection (Figure 3.19a). In NaCl 305 mM, no principal component has actually evolved under the detected multivariate selection coefficients (Figure 3.19b).

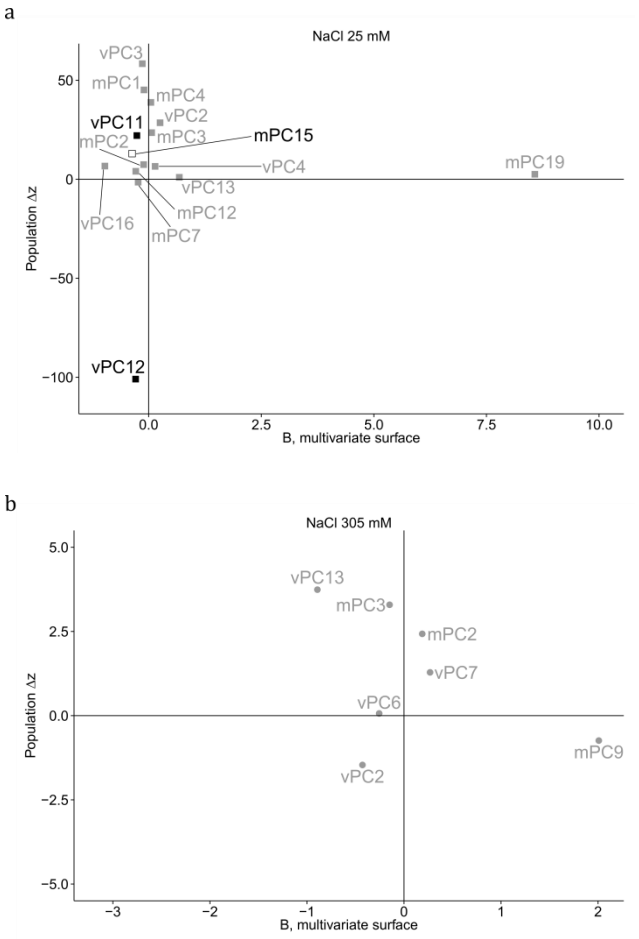


Figure 3.19 – Biplots of the multivariate directional elasticities of selection on principal components of both behaviour centrality and variability (*x*-axis) and their respective evolutionary responses observed in the populations (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). Only the principal components present in the best multivariate selection surface for each environment are present in the respective graphs. The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. The features present the

multivariate selection surfaces for which neither directional selection nor evolution were detected are not plotted.

Selection and evolution seem as congruent when evolutionary responses of the principal components in the inbred lines are under comparison. In NaCl 25 mM, two features have evolved under selection, one of which – median PC12 – in the same direction favoured by selection and the other – median PC19 – in the opposite direction. Furthermore, two other features – median PCs 9 and 15 – have evolved without detectable selection (Figure 3.20a). In NaCl 305 mM, however, there was only one principal component evolving – MAD PC7 – and it did in the direction favoured by selection (Figure 3.20b). In this same environment, there are no evolutionary responses in the principal components that are contrary to the expectation given by the selection coefficients, neither in populations (Figure 3.19b) nor in the inbred lines (Figure 3.20b). Conversely, in NaCl 25 mM, some features have still evolved in the opposite direction to the expected by selection, both in populations (Figure 3.19a) and in the inbred lines (Figure 3.20a). Lastly, in all these multivariate selection surfaces using principal components, the majority of the features under selection did not evolve as a response to that selection (filled datapoints in Figures 3.19 – 3.20).

All in all, in the multivariate selection surfaces using principal components, the features in which evolutionary responses were detected do not match the features for which selection was detected more extensively than in the multivariate selection surfaces using the original features, even though the surfaces using principal components are substantially less collinear than the ones using the original features. Yet, some improvement might be considered in the surfaces in NaCl 305 mM,

because in these no features have evolved in the direction opposite to selection.

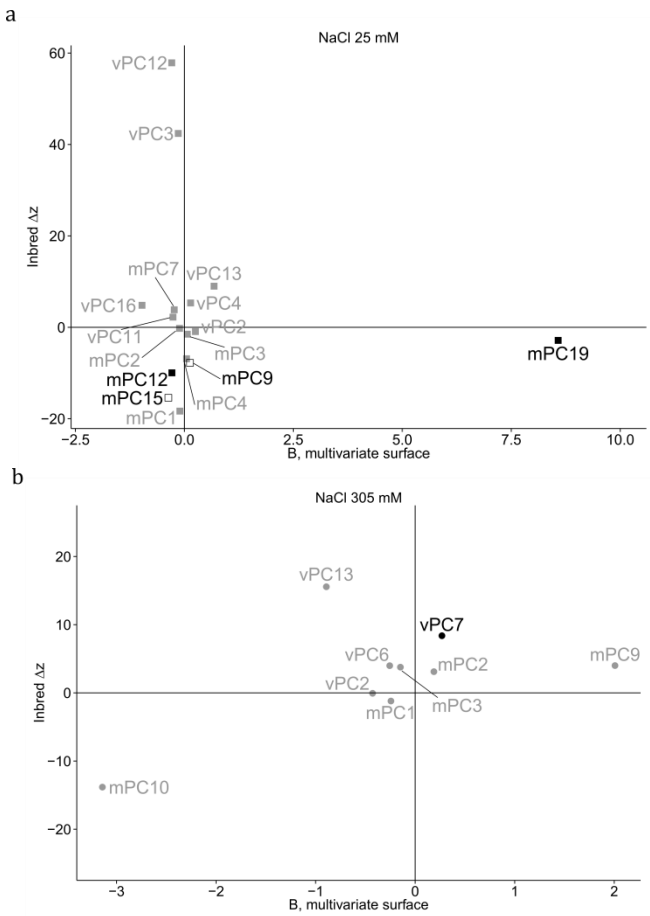


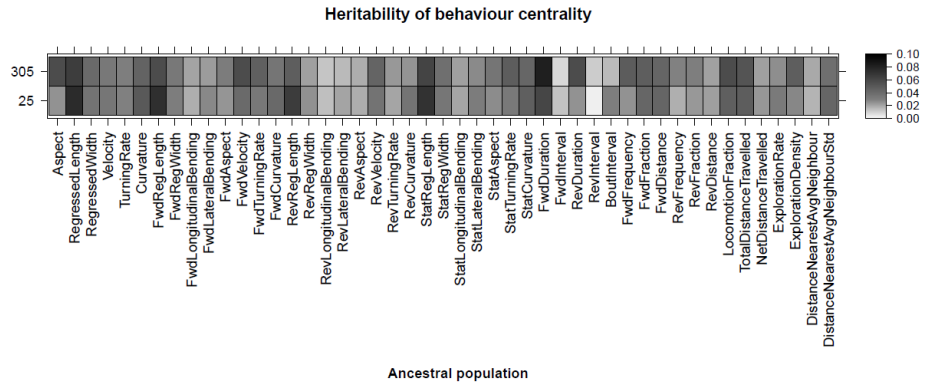
Figure 3.20 – Biplots of the multivariate directional elasticities of selection on principal components of both behaviour centrality and variability (*x*-axis) and their respective evolutionary responses observed in the inbred lines (*y*-axis), both in NaCl 25 mM (**a**) and NaCl 305 mM (**b**). The plots show features under selection (filled squares/circles) and not under selection (open squares/circles), as well as features showing evolutionary response in the populations (black letters) and features that did not evolve (grey letters). Evolutionary responses are measured as a percentage of the respective ancestral values. The features for which no selection direction nor evolution were detected are not represented in the graph.

All original behaviour centrality and variability features show heritability in the ancestral population

Given that evolutionary responses were observed for many features under selection, it is reasonable to check whether the features have heritability, in accordance to Equation 1.7. In order to achieve this purpose, behaviour variation in behavioural centrality and variability features in both NaCl 25 mM and NaCl 305 mM was modelled through a variance components model using the inbred lines derived from the ancestral population. This approach has revealed heritability in all traits under analysis in this thesis, even though their values are low, never higher than 0.1 (Figure 3.20). Furthermore, heritability of behaviour variability is lower than of behaviour centrality features. Specifically, there are particularly low heritabilities, which is the case of interval between reverse locomotion bouts in NaCl 25 mM (Figure 3.20a) and its variability in both environments (Figure 3.20b). These low heritabilities did not seem to impede evolution, for variability in interval between reverse locomotion bouts has evolved in NaCl 25 mM in the populations (Figure 3.11a). In contrast, the highest heritabilities observed concern all regressed length features in NaCl 25 mM and forward locomotion bout duration in NaCl 305 mM. Regarding behaviour variability, the highest heritabilities were observed for velocity, stationary turning rate, forward curvature and forward bout duration (Figure 3.20b).

The presence of heritability in all features under analysis in large populations, along with the existence of selection and evolutionary responses that follow the direction of selection – at least in the direction proposed by univariate selection surfaces – strongly suggests that the the evolving features have responded to natural selection.

a



b

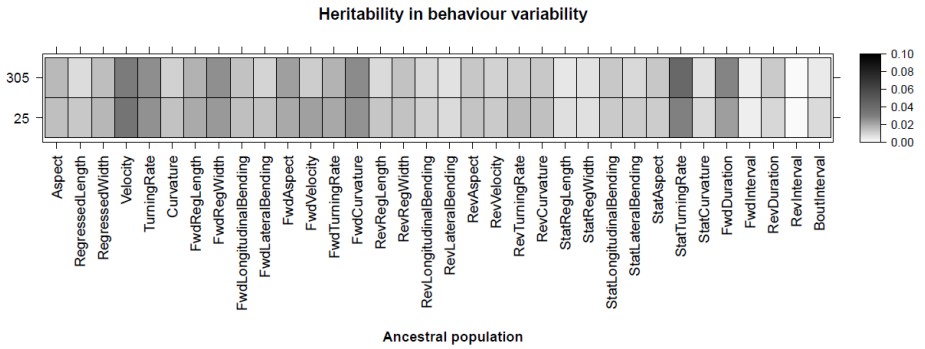


Figure 3.21 – Broad-sense heritabilities of behaviour centrality (a) and variability (b) features in the ancestral population. All values are significantly different from zero. More details on the estimates and their significance are in Appendix 8.

Chapter 4: Discussion

In this thesis, I aimed to understand whether there can be natural selection on behaviour variability besides natural selection on specific behavioural outputs. The question is, for instance, whether locomoting at a specific velocity or with a specific curvature, in which case an animal would engage in a somewhat constant or fixed behavioural strategy, are the only outputs of behaviour that are relevant for individual fitness or, as an alternative, whether locomoting at variable velocities and curvatures can also be relevant for that individual fitness, in which case the animal could explore different strategies in a given period of time or engage in several strategies during that time. If selection for specific behaviour outputs and selection for behaviour variability actually coexist, which of them is the strongest? In other words, which of these behavioural facets – centrality or variability – of a given animal is more relevant to its individual fitness? Moreover, I aimed to assess as well whether a response to this selection can be observed in a novel environment. In such a case, one would also like to assess whether this response was described better by univariate selection surfaces or by a multivariate selection surface.

For these purposes, I have taken advantage of a 50-generation experimental evolution in *C. elegans* in a changing environment using progressively increasing NaCl concentrations among generations. This environmental change was imposed by a changing NaCl concentration; because an abrupt change in NaCl concentration is so impactful that may be lethal (Frazier and Roth, 2009; Lamitina et al., 2004) and, on the contrary, survival is no longer compromised when *C. elegans* individuals are exposed to progressively increasing concentrations of NaCl (Lamitina et al., 2004), I would expect the effects here observed on behaviour would

be virtually unrelated to survival, at least they would not simply represent response to a life-threatening environmental change.

I have measured several behavioural features of kinematic nature, not only in experimental populations in ancestral and evolved state, but also in several inbred lines from derived from each of these experimental populations. On one hand, behavioural measurements in these inbred lines – in particular the inbred lines derived from the ancestral population –, alongside fecundity measurements (used as proxies for fitness), allow the inference of natural selection acting on the ancestral population. Selection was here inferred using both univariate and multivariate selection surfaces. On the other hand, the behavioural measurements in the populations and in inbred lines from those derived allow the detection of evolutionary responses in behaviour and its variability. Lastly, one can compare the patterns of selection and evolution and assess which selection surfaces seem to more adequately describe the evolutionary responses observed.

Both inferences on selection and evolution and evolutionary responses were made in NaCl 25 mM and NaCl 305 mM to take into account the fact that experimental populations have undergone an environment that has progressively changed from NaCl 25 mM to NaCl 305 mM along generations of experimental evolution. The measurements in NaCl 25 mM may serve as an indication of how selection and evolution might have occurred in the onset of experimental evolution, yet, because the environment imposed during experimental evolution on the populations has converged towards NaCl 305 mM –inclusively, the last 15 generations of experimental evolution have taken place in this environment –, I uphold that the measurements of selection and evolution in NaCl 305 mM should describe selection and evolution in this experimental evolution better than the measurements taken in NaCl 25 mM.

Selection has favoured higher locomotory activity and more isolation between individuals

Univariate selection surfaces, by definition, show the total amount of selection exerted on a trait (Walsh and Lynch, 2014), which is the sum of the direct selection on that trait and the indirect selection due to selection on correlated traits (Lande and Arnold, 1983). These selection surfaces have many similarities regarding what I have called behaviour centrality in NaCl 25 mM and in NaCl 305 mM in the ancestral populations, among which lie the selection favouring a higher locomotor activity in general and higher bending; yet, it should be noted, directional selection on longitudinal bendings is stronger in NaCl 305 mM – in some of which selection occurred specifically in this environment (Figure 3.1) – and stabilizing selection on these is also stronger in NaCl 305 mM (Figure 3.2), the one to which experimental evolution has converged. Wave amplitude in an individual *C. elegans* body increases 20-30% during forward locomotion and 35-40% during backward locomotion, during locomotion (Croll, 1975). Hence, it seems likely that directional selection for increased body bending is correlated with the directional selection for increased locomotor activity and an individual *C. elegans* seems to have more fitness by locomoting more. The strong stabilizing selection on longitudinal bending (Figure 3.2) might also be related to this selection on locomotor activity, given that there should be an intermediate optimal level of bending, corresponding to the wave forms the body usually takes when the locomotory waves take place (Croll, 1975). Besides favouring individuals that engage more frequently in locomotion, selection has also favoured individuals that travelled longer distances, yet do not explore larger spaces, for there was no selection for an increase in exploration rate (Figure 3.1). In *C. elegans*, locomotor behaviour in the presence of food has been characterized by mainly two states, one characterized by

low velocity and high turning rate – dwelling – and another characterized by high velocity and lower turning rates, named roaming (Fujiwara et al., 2002). If a behavioural state was favoured by selection, such a state would be an intermediate state between dwelling – given the lower velocity locomotion – and roaming – given the lower turning rate. Alternatively, selection might favour a mixed behavioural strategy in which there is a frequent transition between roaming and dwelling. In this case, the individuals should be engaged more frequently in dwelling than in roaming; this dwelling could mostly consist on back and forth locomotion without much directional change. The ecological value of any of these behavioural strategies is difficult to envisage, nevertheless, because the most basic needs in terms of *C. elegans* life-history seem easily met in this laboratory environment. On one hand, the individuals are overloaded with an excess amount of *E. coli*, a very rich source of food (Shtonda and Avery, 2006). On the other hand, hermaphrodites do not need sexual partners to reproduce, because they can self-fertilize, whereas males do need hermaphrodites to reproduce; hence, selection could eventually favour males that locomote more in order to find hermaphrodites, but not necessarily hermaphrodites that locomote more, especially when this locomotion does not involve larger areas being explored. We know, however, that hermaphrodites increase their locomotion velocity before laying eggs and their reverse bout frequency after laying them (Hardaker et al., 2001). The ecological relevance of these behaviours in context of egg-laying is yet still elusive in the laboratory environment because even though these velocity bursts might facilitate dispersal of egg-laying bursts, there is no obvious need to disperse eggs in order to maximize probability of survival as there seems to be no resource limitation in the laboratory environment.

There was also a clear selection favouring higher distance between individuals, *i.e.* individuals that kept a higher distance to their

conspecifics seem to have higher fitness. This selection might also be correlated with selection for lower velocities, for solitary wild isolates of *C. elegans* were found to move slower than social strains in the presence of food (de Bono and Bargmann, 1998). There is a polymorphism in the G-protein coupled, neuropeptide Y-like receptor NPR-1 that is involved in polymorphism in social behaviour in *C. elegans*; individuals having receptor isoforms with a phenylalanine aminoacid residue in the 215th residue (215F) are social and easily aggregate into clumps of many individuals, whereas individuals having a valine aminoacid residue therein (215V) are solitary (de Bono and Bargmann, 1998). It is known, however, that in the ancestral population used in the context of this thesis, the solitary 215V is the most prevalent isoform, having almost reached fixation even before this ancestral population has come to existence (Teotonio et al., 2012). This evolutionary dynamics seems a natural consequence of the fact that this solitary isoform has more competitive ability than the social, gregarious form 215F in Petri dishes with large homogeneous *Escherichia coli* lawns (Gloria-Soria and Azevedo, 2008). Once no evidence was gathered pointing to effective fixation of this solitary allele in the experimental populations used in this thesis, it is still possible that the gregarious 215F isoform to be still residually present in the experimental populations and selection observed favouring higher distances between individuals and lower velocities to be linked to selection favouring this solitary *npr-1* allele. It is true that other known *loci* are likely targeted by this selection, such as *tyra-3* – coding for a tyramine and octopamine receptor –, but in this case *npr-1* is epistatic over this *locus* and the polymorphism on *tyra-3* brings very little behavioural variation under the solitary *npr-1* allele background (Bendesky et al., 2011). Thus said, in our experimental populations this selection might have low impact on the evolutionary

dynamics of the *tyra-3* polymorphism. Evidently, one cannot deny that *loci* yet to be known might be targeted by this selection as well.

Selection for higher behaviour variability is widespread and relevant on the evolution of behaviour

I expected that when a population is exposed to a novel environment, its individuals would no longer behave optimally. This optimality could be brought from a hard-wired neural network that might have evolved in the previous environment the nematodes were in – NGM NaCl 25 mM, every generation (Teotonio et al., 2012) – and would imply residual learning, if any. In such a case, selection on behaviour variability, if present, would probably favour its decrease, as it would favour more animals that would respond optimally in a more consistent, reliable manner. Conversely, if individuals no longer had optimal behavioural outcomes, they would adjust behaviourally to the novel environment by exploring new behavioural actions and eventually reach to novel optimal behavioural solutions through learning (Sutton and Barto, 1998). In such a scenario, when populations face a novel environment, there would be a selection pressure that would favour individuals that are more capable of incorporating experiences by learning and, because learning rate is higher on individuals that generate more behaviour variability (Wu et al., 2014), we would generate a selection favouring increased behaviour variability.

In fact, behaviour variability was under selection for an increase. Directional selection in univariate surfaces favoured mostly an increase in behaviour variability in what concerns to postural features, which means individuals with more variable body postures along time would have higher fitness. However, the fact that variability in turning rate was selected against suggests as well that postural variability is selectively advantageous, as long as it does not imply massive directional changes along locomotion, especially in what concerns to forward locomotion.

If behaviour features lose variability when they are being consolidated as a result of an action selection process, as it has been observed in the context of motor skill learning (Jin and Costa, 2010; Santos et al., 2015; Wu et al., 2014), then it is possible that some behavioural strategy is being selected that involves a fixed set of orientations during forward locomotion and also given lateral bendings and a given set of orientations during stationary movement. In contrast, selection for increase in variability in the overwhelming majority of the features might indicate that action exploration might be favoured due to lack of action selection. This idea is consistent with the theory that the brain generates variability in behaviour, which might be crucial so that individuals can generate actions with high value, which can eventually be selected (Changeux and Dehaene, 1989; Costa, 2011).

An alternative possibility, however, is that this selection on variability overall might be related to a selection favouring the so-called stomatal oscillations. These are the frequent dorso-ventral oscilations that individuals do with the 10 – 20 μm anteriormost portion of their bodies, which seem to be independent of locomotion and of feeding (Croll 1975). Since the oscillations by themselves do not imply major changes in the main body axis orientation, they may still contribute to a constant direction of movement and therefore to a low variability in turning rate. No detailed study is known on stomatal oscillations and their adaptive

value remains unknown, also because no correlation was found between these oscillations and any other behaviour. I hypothesize that they still are a form of short-sighted exploratory behaviour, because it displaces the head and allows sensilla to sample a larger area. Yet, on one hand, the adaptive value of such short-sighted exploration in this laboratory environment is also elusive; on the other hand, the Multi-Worm Tracker used here (Swierczek et al., 2011) might not have enough resolution to detect the postural changes brought up by these oscillations in each individual so that they could be measured in the behavioural features used here.

The comparison of elasticities of selection between behaviour medians and MADs suggests that selection on behaviour variability might be as strong as on specific behaviours and, even if otherwise, selection on behaviour variability is not at all negligible when compared to selection on specific behaviours. The relevance of selection on variability can also be seen by the fact that the best linear multivariate selection surfaces based either on original features or on principal components have more variability features than centrality features. If we take selection on behaviour variability as selection on exploration or exploitation of behavioural actions and selection on behaviour centrality (median) as selection on given outcomes, the comparison of elasticities of selection also suggests that besides being important that individuals engage in given behavioural actions that might have more value, the ability of individual animals to explore a large range of behavioural actions or exploit a narrow range of actions so that they can find highly valuable ones might also be relevant for their fitness.

It should also be noted that there is also a pervasive stabilizing selection on most behaviour variability features (Figure 3.4; Appendix 3.2), therefore variability is neither endlessly favourable nor unfavourable. Individuals with too low behaviour variability would be too stereotyped

and unable to find actions with higher value and that could decrease fitness. On the other hand, individuals with too high behaviour variability could be unable to select high value actions and that deficient selection could also have fitness costs.

Behaviour and its variability might have evolved under indirect selection

The use of multivariate selection surfaces should bring a more complete picture of selection on all features under analysis. If all traits relevant for fitness are measured, each selection coefficient measured under the multivariate selection surface will measure direct selection on a trait; thus, with such a surface we could distinguish direct from indirect selection (Endler, 1986; Lande and Arnold, 1983; Walsh and Lynch, 2014). Under the same reasoning, I have included both behaviour centrality (median) and variability (MAD) features into the same selection surface; this would allow me to take into account possible effects of scale (Lynch and Walsh, 1998) on variability and say that these are not responsible for the detected selection on behaviour variability.

However, the multivariate selection surface did not succeed in this purpose, for the best linear approximations obtained in both environments (Figure 3.6) were plagued with severe multicollinearity (Tables 3.1 and 3.2). This multicollinearity was substantially ameliorated, but not entirely resolved by principal component analyses (Figures 3.7 and 3.8; Tables 3.3 – 3.6). As a consequence, any given selection coefficient was very unstable and changed dramatically upon exclusion or inclusion of other selection coefficients, as it can be clearly visible as a result of the reduction of the multivariate selection surface using principal components (Appendix 4.4). It is thus unsurprising that the

evolutionary responses observed both in populations and in the inbred lines are much more congruent with the univariate selection surfaces (Figures 3.13 – 3.16) than with the multivariate selection surfaces, regardless of whether the original features (Figures 3.17 – 3.18) or the principal components (Figures 3.19 – 3.20) are used. Nevertheless, it should be noted that the changes in the selection coefficients observed upon reduction of the multivariate selection surfaces using principal components never resulted in sign changes in these coefficients, despite changing statistical significance of many of them (Appendix 4.4). For this reason, the qualitative congruence between natural selection and evolutionary responses would not change for those features that remained statistically significant after reduction of the selection surfaces. Hence, although multicollinearity is so overwhelming in the multivariate selection surfaces using the original features and is likely the main obstacle to obtaining valid selection coefficients, I uphold that there are other reasons apart from multicollinearity that justify the fact that the evolutionary responses match better the univariate selection surfaces than the multivariate counterparts when principal components are used.

By definition, the selection coefficients obtained in the univariate selection surfaces of a trait are the sum of the coefficients of direct selection on that trait and the coefficients of selection on correlated trait, which lead to indirect selection on that trait. Thus said, if a given behavioural trait has evolved due to direct selection, one would observe congruence between selection and evolution both in univariate surfaces using this trait and multivariate surfaces including this focal trait, then the direction of selection and evolution would be congruent both in univariate and multivariate surfaces. However, because this scenario can occur any instance in which both univariate and the respective

multivariate selection coefficients have the same sign, this congruence in both selection surfaces does not strictly imply neither that direct selection on the trait is the only selection taking place on the trait, nor that it is the major selection force in place; it is also possible that the sum of correlated selection coefficients has the same sign of the direct selection coefficient and therefore the total selection will have the same sign of the direct selection coefficient. To make this entirely clear, as only the sum of the indirect selection coefficients needs to have the same sign of the univariate selection coefficient, one can find many individual indirect selection coefficients of both signs and the aforementioned condition still hold.

Here, the comparison between the univariate and respective multivariate selection coefficients demands extreme caution, due to the multicollinearity observed. Nevertheless, by comparing, in NaCl 305 mM, the previously the directional selection coefficients from the univariate (Figure 3.3b) and multivariate (Figure 3.6b) selection surfaces, we could envisage the possibility that variability in aspect during reverse locomotion was under direct selection in the environment to which experimental evolution has converged. The same comparison using the multivariate selection surfaces from principal components is more difficult to make, for the principal components and the original features are not directly comparable. Nevertheless, if we think of the principal components has a way forward to leap over multicollinearity and keep the original features in mind, we can have a glimpse on which traits would virtually be under direct selection by looking at the eigenvectors that gave rise to those principal components in the respective environments (Appendix 4.5). For instance, in the case of NaCl 305 mM, in the inbred lines, MAD PC7 was the principal component that has evolved in the direction expected under selection (Figure 3.20b). The major principal component loadings in MAD PC7 in NaCl 305 is by far

overall regressed width, with a positive loading; other features have also a substantial loading when compared to the previously mentioned one, such as stationary longitudinal bending – with a positive loading as well – , interval between bouts, stationary, overall and forward curvatures, velocity and longitudinal bending during reverse locomotion – with negative loadings (Appendix 4.5). In other words, this principal component is a linear combination of all behaviour variability features present in the multivariate selection surface in NaCl 305 mM (Figure 3.6b), to which the aforementioned features are the main contributors. Yet, this is not equivalent to say we could observe partial selection on the features with the major loadings in the direction given their signs. Instead, when the principal components are obtained, one is dealing with new features, therefore the loadings of the original features will eventually help us to interpret what the new features actually represent biologically.

A more detailed look to the multivariate selection surfaces using principal components – ignoring, at this point, their biological interpretation – can still point to the importance of direct natural selection in the evolution of behaviour and its variability in the experimental populations. It is interesting to note that most of the features in both environments, both in populations (Figure 3.19) and inbred lines (Figure 3.20) show selection without evolution. Because we are looking at selection in a multivariate selection surface, the selection observed on these features is somehow a direct selection, or at least selection which takes into account the indirect selection on the focal trait that is due to the correlation with other measured traits in this thesis. Thus said, what is observable in these multivariate selection surfaces is that most of the principal components do not respond to direct selection.

Two explanations can be devised to justify this lack of response to selection. The first is that the principal components might have no heritability (see Equation 1.7). The hypothetical absence of heritability may also justify the lack of response to selection observed in the univariate selection surfaces (Figures 3.15 – 3.16). The second explanation for the lack of response to selection is that there might have been correlated traits, which were not measured, under selection as well and therefore the direct selection coefficients here estimated may be biased by those correlated, unmeasured traits. Such bias would occur because the regressions used for the multivariate selection surface assume all relevant traits to fitness were measured (Lande and Arnold, 1983) and such assumption would not hold. When this assumption is violated, the residuals of the ordinary least squares regression used are not be independent from the focal trait to which the unmeasured traits are correlated (Mitchell-Olds and Shaw, 1987).

I recognize that some principal components here obtained might have an undetectable heritability, especially the principal components that carry the lowest variances – typically the latest ones, once principal components analysis retrieves orthogonal components with decreasing variances (Jolliffe, 2005). However, I would argue that many, if not most, of these principal components do have heritability, because they were obtained as linear combinations of the original traits, all of which show broad-sense heritability (Figure 3.21). It is true that broad-sense heritability is an upper bound approximation of narrow-sense heritability, the latter being required for a response to selection (Equation 1.7). Notwithstanding, we observe evolutionary responses in behaviour centrality (Figure 3.9) and behaviour variability features (Figure 3.11), which highly suggests that these features also have narrow-sense heritability. Furthermore, many principal components result from linear combinations of features that have shown evolutionary

response. This is especially evident in the case of behaviour variability principal components used for the multivariate selection surfaces in NaCl 305 mM, which are linear combinations of 13 features (Appendix 4.5), 8 of which – regressed width overall and during forward locomotion, curvature and aspect during forward locomotion, longitudinal bending, lateral bending and aspect during backward locomotion and duration of reverse locomotion bouts – have shown evolutionary response congruent with univariate selection both in populations (Figure 3.15) and in inbred lines (Figure 3.16). Therefore, I hypothesize that most of these behaviour centrality and variability principal components did not respond to multivariate selection because they were under indirect selection due to correlation with unmeasured trait relevant for fitness. Furthermore, given that it is difficult to find a reasonable adaptive value on an increase in locomotory rate in such a homogenous, food-filled laboratory environment, or an increase in behaviour variability without a visible increase in area exploration rate, I extend this hypothesis to the original behavioural features as well.

The question now is which features that were not measured could be under selection. I strongly uphold that if the traits under direct selection in the experimental evolution were not of behavioural nature, then they might likely be of physiological nature. Since the environment imposed in the experimental evolution increased progressively in osmolarity – coming from a NaCl concentration of 25 mM to 305 mM and subsequent maintenance of NaCl concentration in this level (Theologidis et al., 2014) –, there might have been a strong selective pressure on physiological mechanisms of osmotic stress response and selection for behaviour variability might have been correlated with direct selection for physiological responses to osmotic stress. This selective pressure comes from the fact that exposure to hyperosmotic stress leads to substantial water loss (Lamitina et al., 2004) and it is hypothesized to lead as well to

extensive protein damage and misfolding due to the increased ionic strength in the cytoplasm of each cell (Lamitina et al., 2006), which may trigger the synthesis of organic osmolytes, the main of which will be glycerol (Lamitina et al., 2004).

I suggest that during experimental evolution – especially when it comes to the highest concentrations of NaCl –, turgor pressure in individual bodies has decreased chronically and therefore the ionic strength of all body cells, including neurons, has chronically increased throughout evolution. As a consequence, the number of functional protein molecules would be lower than it was in the ancestral population in NaCl 25 mM, for a part would be damaged.

If we think about ion channels in neurons, for instance, selection might have occurred on the level of synthesis and favour individuals with lower protein synthesis. By lowering the amount of protein synthesis, the cytoprotective effect of glycerol (Yancey, 2005; Yancey et al., 1982) might be more effective because the number of glycerol molecules an individual nematode is able to produce is limited and the lesser the number of proteins in the cell, the lesser the amount of glycerol should be needed to avoid protein damage and misfolding. The energy saved by the decrease in protein synthesis could be reallocated for reproduction by production of more eggs. This trade-off leveraging is plausible given that each individual has to invest a lot of its glycogen resources in order to produce glycerol and maintain an acceptable turgor pressure inside their bodies when facing osmotic stress (Frazier and Roth, 2009). Consequently, behaviour variability would be selected by correlation, indirectly, because a lower amount of ion channels would lead to higher variability in action potential timing (Schneidman et al., 1998), which could likely lead to variability in the behavioural output (Renart and Machens, 2014). In order to test such hypothesis, protein synthesis could be quantified in

pools of individual nematodes from the experimental populations in generations 0 and 50, in NaCl 25 mM and NaCl 305 mM as well. This quantification could be done through by protein-labelling; individuals would be fed with *E.coli* labelled with nitrogen-15 (^{15}N) and this nitrogen isotope would be incorporated in the individual nematode's proteins. Later, one can pool populations and quantify protein using mass spectrometry (Krijgsveld et al., 2003).

In this indirect selection scenario, selection and evolution of behaviour variability here observed do not seem to uphold a selectionist theory of learning new actions (Changeux and Dehaene, 1989; Costa, 2011), but such a theory cannot yet be excluded because direct and indirect selection could still coexist. For a direct demonstration or exclusion of this theory, learning rate of inbred lines with different behaviour variabilities should be assessed. Learning rate would ideally be assessed by engaging individuals in an operant conditioning task, in which individuals undergo a task that gives them a feedback as an outcome of their actions, which can range from reinforcement to punishment (Skinner, 1938). In such an experimental setup, we can devise a brand new task we would like individuals to learn. No operant learning task has yet been developed in *C. elegans*, but it seems technically feasible to design an operant task with reinforcement possibly based on direct neural activation of dopaminergic neurons (Schultz et al., 1997) contingent on the performance of a given behavioural pattern; such an experiment should evidently be done in a Petri dish without food, as food would work as a confounding reinforcer. Such an experiment might be possible in the near future, as technology has been bringing the possibility of optogenetic activation of specific cells with high time resolution (Leifer et al., 2011; Stirman et al., 2011). As an alternative, learning rate could be assessed using a classical conditioning task, using odours for instance. In this setting, we could pair odours that are noxious

in naïve animals with food and check how quickly the individuals would change their preferences towards the previously noxious stimuli (Bargmann and Horvitz, 1991). In such assays, behaviour variability, according to the selectionist hypothesis, would allow the individuals to find the odours paired with the reinforcer more quickly as they engage more often in exploratory behaviour.

Behaviour variability here is actually a form of within-individual variation. Components of variation existing within a given individual have been studied mostly using fluctuating asymmetry as a phenotype. Fluctuating asymmetry is the difference between corresponding phenotypes on the left and right side of bilaterally symmetric individuals, *e.g.* left and right wing asymmetry in fruit flies (Leamy and Klingenberg, 2005). Selection on fluctuating asymmetry has been shown in some experiments involving sexual selection, which have shown that individuals with higher fluctuating asymmetry have lower mating success (Martín and López, 2000; Santos, 2001). Although experiments involving crosses between inbred lines with different asymmetry have shown genetic influences on fluctuating asymmetry (Mather, 1953), either its heritability is also low (Carter and Houle, 2011) or that genetic influence is not apparent in all species (Santos, 2001). Theoretical work has also been done on within-individual variation in plant flowering time, which raises the hypothesis that pollinator attraction or a short reproductive season might lead to a decrease in within-individual variance in flowering time and environmental stochasticity of pollinator visitation might increase that within-individual variation (Devaux and Lande, 2010). In this context, this thesis adds experimental data that point to the existence of selection on heritable within-individual variation on behaviour and its evolution as well.

Sexual dimorphism and inbreeding effects are likely on behaviour and its variability

One inescapable observation in this dataset is that the evolutionary responses observed in the experimental populations themselves are different from the evolutionary responses observed in inbred lines that were derived from those experimental populations. This difference is very striking in behaviour centrality (Figure 3.9) – in which there are many more features evolving in the inbred lines than in the populations –, and visible to a lower extent in behaviour variability (Figure 3.11) – in which there are features only evolving in the populations, but also some features only evolving in the inbred lines. The fact that overwhelming majority of the features have evolutionary responses are of the same sign in populations and inbred lines in NaCl 305 mM highly suggests that these differences come from statistical power issues. In accordance to this possibility, the number of individual nematodes tracked in the inbred lines is of a different order of magnitude of the number tracked in the populations (Appendix 2). A second reason accounting for differences between population and inbred line behavioural values is that populations are composed of hermaphrodites and males, whereas inbred lines are made of hermaphrodites and males only rarely appear by spontaneous X chromosome non-disjunction (Herman, 2005). Behavioural differences could exist because we are observing males in the populations and not in the inbred lines and behavioural differences are quite striking between *C. elegans* hermaphrodites and males (Portman, 2007). Although male frequency has decreased sharpedly in the populations during experimental evolution (Theologidis et al., 2014), male frequency in tracking plates is similar in generations 0 and 50 in both environments (Appendix 7), therefore not only the evolutionary responses observed in the populations cannot be explained by male

frequency itself, but also male frequency is out of the equation in what concerns to evolutionary response differences between populations and inbred lines. A third reason accounting for the behavioural differences between populations and inbred lines is the presence of non-additive genetic effects. If we fairly assume that the genetic pool is equivalent in the populations and in the inbred lines, it seems likely that the differential distribution of alleles along the inbred lines changes the expression of the behaviours in the whole set of inbred lines when compared to the populations. This might mean that different combinations of alleles within and among *loci* lead to different behavioural outcomes, *i.e.* there is non-additive genetic variation for these features. Non-additive genetic effects could have greatly influenced the evolution of the features that did not share the same trend in inbred lines and populations, especially in NaCl 25 mM (Figures 3.10a and 3.12a). These non-additive effects might also have contributed to the expression of phenotypes from deleterious alleles in the inbred lines – thereby bringing inbreeding depression (Charlesworth and Charlesworth, 1987) – that could also affect the value of behavioural features. Inbreeding depression has been observed in the ancestral population (Chelo et al., 2014) and also in the evolved populations here used (Chelo et al., in prep.). Furthermore, it seems that the degree of inbreeding depression is lower in the evolved populations than in the ancestral population (Chelo et al., in prep.). Therefore, both inbreeding depression and its evolution on these populations might account for a large fraction of the differences between populations and inbred lines, especially those observed in NaCl 25 mM (Figures 3.10a and 3.12a).

While the statistical power issues could eventually be addressed by a dramatic increase in the number of individuals tracked in the populations, the biological possibilities can be addressed in a more pragmatic manner and bring useful biological knowledge. To assess the

importance of males in the differences observed, one could track only hermaphrodites coming from the populations and directly compare them both with the original populations – with the hermaphrodites and males – and the inbred lines. On the other hand, to assess whether the behavioural features have non-additive genetic effects, we could cross individual nematodes coming from inbred lines showing opposite extreme values of the behavioural features and track the progeny and compare their behaviour with the behaviour of the parental inbred lines. If the behavioural features are additive, one should observe a distribution of behaviour values that lies between parental values and should peak in the mid-value of these parents (Falconer and Mackay, 1995; Fisher, 1918; Lynch and Walsh, 1998). Striking deviations of this pattern would show non-additive genetic effects on these behaviours.

Final remarks

This thesis shows that both behaviour and behaviour variability are under widespread selection in a context of experimental evolution on a changing environment and that selection on behaviour variability is as relevant as selection on specific behavioural outputs. Also, this thesis has shown that, in this environmental setting, evolutionary responses in behaviour and its variability were also widespread and, notably, behaviour variability has mostly increased. However, this evolutionary response is likely the result of indirect selection by correlation with selection on an unmeasured trait that may not be behavioural.

References

Bargmann, C.I., and Horvitz, H.R. (1991). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. Neuron *7*, 729–742.

Bendesky, A., Tsunozaki, M., Rockman, M.V., Kruglyak, L., and Bargmann, C.I. (2011). Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. Nature *472*, 313–318.

de Bono, M., and Bargmann, C.I. (1998). Natural Variation in a Neuropeptide Y Receptor Homolog Modifies Social Behavior and Food Response in *C. elegans*. Cell *94*, 679–689.

Carter, A.J.R., and Houle, D. (2011). Artificial Selection Reveals Heritable Variation for Developmental Instability. Evolution *65*, 3558–3564.

Changeux, J.P., and Dehaene, S. (1989). Neuronal models of cognitive functions. Cognition *33*, 63–109.

Charlesworth, D., and Charlesworth, B. (1987). Inbreeding Depression and its Evolutionary Consequences. Annu. Rev. Ecol. Syst. *18*, 237–268.

Chelo, I.M., Carvalho, S., Roque, M., Proulx, S.R., and Teotónio, H. (2014). The genetic basis and experimental evolution of inbreeding depression in *Caenorhabditis elegans*. Heredity *112*, 248–254.

Chelo, I.M., Carvalho, S., Afonso, B.C., Theologidis, I., Goy, C., and Teotónio, H. Shaping genetic diversity during adaptation to a new environment in experimental *Caenorhabditis elegans* populations with different mating systems. Prep.

Costa, R.M. (2011). A selectionist account of *de novo* action learning. Curr. Opin. Neurobiol. *21*, 579–586.

Croll, N.A. (1975). Components and patterns in the behaviour of the nematode *Caenorhabditis elegans*. J. Zool. *176*, 159–176.

Devaux, C., and Lande, R. (2010). Selection on Variance in Flowering Time Within and Among Individuals. Evolution *64*, 1311–1320.

Endler, J.A. (1986). Natural Selection in the Wild (Princeton University Press).

Falconer, D.S., and Mackay, T.F.C. (1995). Introduction to Quantitative Genetics (Essex, England: Longman).

Fisher, R.A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. Trans. R. Soc. Edinb. *52*, 399–433.

Frazier, H.N., and Roth, M.B. (2009). Adaptive Sugar Provisioning Controls Survival of *C. elegans* Embryos in Adverse Environments. Curr. Biol. *19*, 859–863.

Fujiwara, M., Sengupta, P., and McIntire, S.L. (2002). Regulation of Body Size and Behavioral State of *C. elegans* by Sensory Perception and the EGL-4 cGMP-Dependent Protein Kinase. Neuron *36*, 1091–1102.

Gloria-Soria, A., and Azevedo, R.B.R. (2008). *npr-1* Regulates Foraging and Dispersal Strategies in *Caenorhabditis elegans*. Curr. Biol. *18*, 1694–1699.

Hardaker, L.A., Singer, E., Kerr, R., Zhou, G., and Schafer, W.R. (2001). Serotonin modulates locomotory behavior and coordinates egg-laying and movement in *Caenorhabditis elegans*. J. Neurobiol. *49*, 303–313.

Herman, R. (2005). Introduction to sex determination. WormBook.

Jin, X., and Costa, R.M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature *466*, 457–462.

Jolliffe, I. (2005). Principal Component Analysis. In *Encyclopedia of Statistics in Behavioral Science*, (John Wiley & Sons, Ltd), p.

Krijgsveld, J., Ketting, R.F., Mahmoudi, T., Johansen, J., Artal-Sanz, M., Verrijzer, C.P., Plasterk, R.H.A., and Heck, A.J.R. (2003). Metabolic labeling of *C. elegans* and *D. melanogaster* for quantitative proteomics. Nat. Biotechnol. *21*, 927–931.

Lamitina, S.T., Morrison, R., Moeckel, G.W., and Strange, K. (2004). Adaptation of the nematode *Caenorhabditis elegans* to extreme osmotic stress. Am. J. Physiol. - Cell Physiol. *286*, C785–C791.

Lamitina, T., Huang, C.G., and Strange, K. (2006). Genome-wide RNAi screening identifies protein damage as a regulator of osmoprotective gene expression. Proc. Natl. Acad. Sci. *103*, 12173–12178.

Lande, R., and Arnold, S.J. (1983). The Measurement of Selection on Correlated Characters. Evolution *37*, 1210.

Leamy, L.J., and Klingenberg, C.P. (2005). The Genetics and Evolution of Fluctuating Asymmetry. Annu. Rev. Ecol. Evol. Syst. *36*, 1–21.

Leifer, A.M., Fang-Yen, C., Gershow, M., Alkema, M.J., and Samuel, A.D.T. (2011). Optogenetic manipulation of neural activity in freely moving *Caenorhabditis elegans*. Nat. Methods *8*, 147–152.

Lynch, M., and Walsh, B. (1998). Genetics and Analysis of Quantitative Traits (Sunderland, Mass.: Sinauer Associates Inc.,U.S.).

Martín, J., and López, P. (2000). Chemoreception, symmetry and mate choice in lizards. Proc. R. Soc. B Biol. Sci. *267*, 1265–1269.

Mather, K. (1953). Genetical control of stability in development. Heredity *7*, 297–336.

Mitchell-Olds, T., and Shaw, R.G. (1987). Regression Analysis of Natural Selection: Statistical Inference and Biological Interpretation. Evolution *41*, 1149–1161.

Portman, D.S. (2007). Genetic Control of Sex Differences in *C. elegans* Neurobiology and Behavior. B.-A. in Genetics, ed. (Academic Press), pp. 1–37.

Renart, A., and Machens, C.K. (2014). Variability in neural activity and behavior. Curr. Opin. Neurobiol. *25*, 211–220.

Santos, M. (2001). Fluctuating Asymmetry Is Nongenetically Related to Mating Success in *Drosophila buzzatii*. Evolution *55*, 2248–2256.

Santos, F.J., Oliveira, R.F., Jin, X., and Costa, R.M. (2015). Corticostriatal dynamics encode the refinement of specific behavioral variability during skill learning. eLife e09423.

Schneidman, E., Freedman, B., and Segev, I. (1998). Ion Channel Stochasticity May Be Critical in Determining the Reliability and Precision of Spike Timing. Neural Comput. *10*, 1679–1703.

Schultz, W., Dayan, P., and Montague, P.R. (1997). A Neural Substrate of Prediction and Reward. Science *275*, 1593–1599.

Shtonda, B.B., and Avery, L. (2006). Dietary choice behavior in *Caenorhabditis elegans*. J. Exp. Biol. *209*, 89–102.

Skinner, B.F. (1938). The behavior of organisms: an experimental analysis (Oxford, England: Appleton-Century).

Stirman, J.N., Crane, M.M., Husson, S.J., Wabnig, S., Schultheis, C., Gottschalk, A., and Lu, H. (2011). Real-time multimodal optical control of neurons and muscles in freely behaving *Caenorhabditis elegans*. Nat. Methods *8*, 153–158.

Sutton, R.S., and Barto, A.G. (1998). Reinforcement Learning: An Introduction (MIT Press).

Swierczek, N.A., Giles, A.C., Rankin, C.H., and Kerr, R.A. (2011). High-throughput behavioral analysis in *C. elegans*. Nat. Methods *8*, 592–598.

Teotonio, H., Carvalho, S., Manoel, D., Roque, M., and Chelo, I.M. (2012). Evolution of Outcrossing in Experimental Populations of *Caenorhabditis elegans*. PLoS ONE *7*, e35811.

Theologidis, I., Chelo, I.M., Goy, C., and Teotónio, H. (2014). Reproductive assurance drives transitions to self-fertilization in experimental *Caenorhabditis elegans*. BMC Biol. *12*, 93.

Walsh, B., and Lynch, M. (2014). Genetics and Analysis of Quantitative Traits, Volume 2: Evolution and Selection of Quantitative Traits.

Wu, H.G., Miyamoto, Y.R., Castro, L.N.G., Ölveczky, B.P., and Smith, M.A. (2014). Temporal structure of motor variability is dynamically regulated and predicts motor learning ability. Nat. Neurosci. *17*, 312–321.

Yancey, P.H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J. Exp. Biol. *208*, 2819–2830.

Yancey, P., Clark, M., Hand, S., Bowlus, R., and Somero, G. (1982). Living with water stress: evolution of osmolyte systems. Science *217*, 1214–1222.

Appendix 1: Best model equations for behaviour MAD as a function of sampling time.

These equations refer to the models shown in Figure 2.11 of the thesis. These were the equations used for derivation. In the equations here, t is sampling time, l' is length MAD and v' is velocity MAD; subscripts state the environment, defined by NaCl concentration. None of these models could be reduced from the starting model stated in Equation 2.3, as reduction would always increase AIC (see Equation 2.4).

$$l_{25}' = 0.0004925665\ t + 0.0254099227\ \log t - 0.0139211944\ \sqrt{t}$$

$$l_{305}' = 0.0004724759\ t + 0.0237623734\ \log t - 0.0132806206\ \sqrt{t}$$

$$v_{25}' = 0.00040551\ t + 0.01876691\ \log t - 0.01136841\ \sqrt{t}$$

$$v_{305}' = 0.0003866352\ t + 0.0186576137\ \log t - 0.0109767697\ \sqrt{t}$$

Appendix 2: Nematode track and individual sample sizes.

Number of inbred lines (population of origin):

311 = 180 (A6140) + 50 (G50A1) + 51 (G50A2) + 30 (G50A4).

Appendix 2.1: Sample sizes associated to each population in each environment.

Population	Number of individuals		Number of tracks	
	NaCl 25 mM	NaCl 305 mM	NaCl 25 mM	NaCl 305 mM
G50A4	3518	3443	7687	6327
G50A1	1995	2733	3552	4804
G50A2	2723	4113	4921	6760
140A6	1975	2144	3522	4434
Total	10211	19682	12649	22325

Appendix 2.2: Sample sizes associated to each inbred line in each environment.

Inbred line	Number of individuals		Number of tracks	
	NaCl 25 mM	NaCl 305 mM	NaCl 25 mM	NaCl 305 mM
G50A1L30	707	514	1174	832
A6140L309	1003	1233	1952	1721
A6140L349	738	844	1279	1210
A6140L13	763	702	1682	1124
G50A4L23	1021	631	1997	1126
G50A2L24	833	1343	1119	1488
G50A1L28	1319	1119	2140	2835
G50A2L25	1181	1526	2161	2350
A6140L49	1200	986	1745	1777
A6140L287	890	1004	1539	1609
A6140L203	528	315	1475	891
G50A1L10	793	1010	1737	2040
A6140L150	1144	1251	1703	1604
A6140L19	785	884	1881	1712
G50A4L25	1484	1936	2699	2833
G50A2L6	1354	1171	1908	1706
A6140L183	962	377	2266	717
A6140L184	1141	1294	2249	2329
G50A4L22	1001	1090	1795	1755
A6140L228	756	898	1439	1728
A6140L182	1097	898	1654	1591
A6140L218	482	838	901	1288
G50A2L13	880	1779	1966	2481
G50A1L40	567	866	1538	1823
G50A4L21	661	895	1057	1416
A6140L289	681	361	1419	875
A6140L272	1081	1336	1517	1932
G50A1L7	638	727	2023	1956
G50A2L27	1053	717	2115	1419
G50A4L27	747	1055	2228	2191
G50A2L19	912	989	1675	1930
A6140L236	1169	885	2446	1534

G50A2L18	953	1053	1791	2160
A6140L48	814	1088	1205	1912
G50A1L6	1154	2065	2384	2796
G50A1L33	922	1174	1429	1756
G50A4L17	1007	1474	1704	2125
A6140L293	547	291	1408	585
G50A1L8	1163	1758	1668	2314
G50A2L20	873	1838	1227	2174
G50A1L24	777	1086	1630	1786
A6140L257	871	396	1535	746
A6140L333	573	823	1080	1618
G50A2L17	1010	870	2003	1539
G50A1L29	925	920	1378	1399
A6140L211	857	894	1725	1417
G50A1L35	852	1249	1843	1878
A6140L133	830	1299	1565	2028
G50A1L4	904	1229	1869	1938
A6140L200	767	753	1157	1212
A6140L181	1169	1486	2482	2211
A6140L110	888	844	1775	1621
A6140L265	945	1641	1525	2304
G50A4L20	881	662	1626	1165
A6140L18	837	1249	1597	1995
G50A1L25	924	1780	1539	2079
A6140L282	612	824	1226	1486
G50A4L9	852	1175	1637	1913
A6140L82	926	911	1559	1490
G50A1L23	934	1591	2442	2833
A6140L155	930	1159	1574	1746
A6140L283	1077	1288	2195	2130
G50A1L18	875	1124	2027	1694
G50A2L26	663	885	1412	1461
G50A1L22	547	803	1309	1949
A6140L113	678	525	968	867
G50A4L15	653	1492	1185	2072
G50A4L2	972	1014	1585	1526
A6140L106	737	1203	1320	1989

A6140L137	1656	1035	3350	2837
A6140L134	986	980	1488	1479
A6140L261	941	608	1585	905
A6140L357	882	674	1621	1007
G50A4L14	740	1540	1238	1957
A6140L60	808	892	1106	1580
G50A1L26	1084	1309	1818	2185
G50A2L10	1081	977	1762	1528
A6140L271	433	268	1090	491
G50A4L13	651	1058	1649	1984
A6140L205	1063	835	2051	1508
G50A2L7	928	1185	1890	1721
A6140L326	763	1112	1978	1973
G50A2L22	1455	1252	2377	2065
A6140L107	1384	1456	2311	2394
G50A2L2	962	1344	2003	1972
G50A4L6	1102	1029	2609	1651
G50A2L4	1113	1770	2514	2507
G50A2L21	489	1091	967	1990
G50A2L30	1026	1496	2042	2364
G50A1L34	1020	1133	2201	1809
A6140L351	556	132	1706	426
A6140L324	599	788	1577	1482
A6140L135	934	1368	1406	1962
A6140L290	965	1147	1381	1665
G50A1L39	1039	646	2480	1661
G50A2L29	915	1253	1946	1636
G50A4L10	1021	663	1516	976
G50A4L24	498	621	1296	1264
A6140L234	826	347	2193	916
G50A4L29	1057	1370	2884	2024
A6140L273	669	790	1169	1471
G50A1L3	659	1005	1318	1734
G50A2L28	981	1459	1779	2120
G50A2L3	986	1316	1818	2439
A6140L258	925	968	1520	1775
G50A2L1	842	1331	2083	2095

A6140L120	770	1250	1660	2037
G50A2L11	740	1228	1821	1743
G50A1L27	1017	343	1599	572
A6140L63	901	1095	1821	1635
A6140L112	913	726	2059	1546
G50A1L20	769	534	1626	1154
G50A1L17	1202	1404	2465	2189
A6140L123	842	617	2116	1234
A6140L176	291	293	443	432
G50A2L14	1161	1023	2614	2079
A6140L250	862	987	1325	1442
G50A1L36	1171	923	2496	1778
G50A2L9	1058	666	2028	1035
A6140L294	758	805	1376	1495
A6140L188	1209	856	1817	1497
A6140L124	969	625	1534	1254
G50A4L3	1006	1156	1629	1783
G50A1L11	1449	1642	2140	2518
A6140L225	848	492	1485	1171
G50A1L21	1067	1018	1406	1488
G50A1L12	922	930	1422	1247
G50A4L30	894	1421	1169	1851
G50A4L28	879	716	1153	985
A6140L252	1019	1291	2059	1978
G50A4L1	925	1217	2121	1787
A6140L233	929	1084	1501	1688
A6140L247	1245	1490	2092	2044
A6140L226	792	740	2003	1436
G50A2L12	875	830	1309	1217
A6140L157	915	1076	2456	2081
G50A4L8	680	382	1368	558
A6140L239	713	649	1527	1251
G50A4L26	1034	1090	1731	1686
G50A2L8	667	1160	1261	1553
A6140L179	766	1087	1223	1844
G50A1L2	670	1078	1521	1531
G50A1L19	924	653	1905	1175

G50A4L18	972	1018	1873	2045
G50A4L5	1089	1483	2125	2287
A6140L253	779	765	1331	1086
G50A1L16	1095	927	2262	1781
A6140L217	775	1002	1541	1946
G50A2L5	875	1367	1857	2210
G50A2L16	851	1170	1803	2170
A6140L201	618	1029	1051	1670
A6140L142	778	650	2470	1616
G50A1L1	1014	850	2957	1915
G50A4L11	1205	1546	1823	2305
A6140L331	728	821	1825	1708
G50A1L14	881	834	1905	1780
A6140L125	778	588	1762	1550
A6140L246	640	618	1005	1326
G50A1L5	794	529	1314	714
A6140L28	1282	1585	2218	2791
G50A1L37	729	897	2047	2230
A6140L158	841	718	1606	1465
A6140L264	912	1265	1726	2123
A6140L337	925	1207	1755	2436
G50A1L32	792	998	1625	1931
A6140L122	884	916	1681	1587
A6140L350	346	188	702	534
A6140L353	1008	1189	1241	1775
A6140L175	949	976	1503	1459
G50A1L13	680	452	1192	630
G50A2L15	754	1858	1672	2433
A6140L244	824	619	1804	1205
G50A4L7	1186	1437	2237	1925
A6140L169	676	639	2116	1653
A6140L266	793	313	1409	813
G50A1L38	980	1385	1650	2155
A6140L231	548	390	1151	744
A6140L268	701	774	855	1148
A6140L248	514	436	1323	1010
G50A1L31	623	893	1694	2026

A6140L56	634	704	1338	1414
A6140L251	710	1101	1493	1843
G50A4L4	934	950	1919	1820
A6140L156	611	770	881	1288
G50A1L15	1134	910	2544	2205
A6140L168	801	560	1793	981
A6140L153	921	731	1644	1598
A6140L317	456	327	972	607
A6140L355	591	471	1568	993
G50A4L12	777	1046	1824	2025
G50A4L19	1131	1249	2353	1738
A6140L126	354	408	864	845
G50A1L46	522	420	821	841
G50A2L48	758	1471	2244	2444
G50A1L49	1343	1719	2623	2711
A6140L94	926	1226	1563	1968
G50A1L43	878	1093	2506	2198
G50A2L47	1062	1528	1709	2142
A6140L109	736	993	1377	1770
G50A2L49	947	1469	1745	1974
A6140L105	863	1265	1491	2158
G50A2L42	1442	1512	2121	1812
A6140L58	700	562	1382	1026
A6140L73	688	723	1229	1176
G50A1L42	765	1128	1347	1588
G50A2L36	1537	1320	2269	1765
A6140L31	771	1134	1322	1749
A6140L11	557	468	1422	1049
A6140L37	595	618	1762	1480
A6140L29	676	798	1188	1519
A6140L67	477	507	1111	1045
A6140L10	463	545	764	887
A6140L17	446	449	1184	730
G50A2L50	1242	1300	2134	2101
A6140L65	785	645	1272	1279
A6140L59	684	483	1337	1094
A6140L44	470	424	776	758

A6140L20	396	702	774	1190
A6140L51	838	807	1465	1132
A6140L7	767	1602	1222	2212
G50A2L58	1452	1284	2983	2170
G50A2L40	820	1823	1787	2496
G50A1L48	876	569	2419	1083
A6140L81	250	218	547	426
A6140L12	874	201	1718	336
A6140L93	406	168	624	320
A6140L14	645	281	1410	602
A6140L5	460	463	802	702
A6140L97	805	818	2024	1645
A6140L2	1021	1498	1768	2414
A6140L115	856	954	1797	1576
A6140L40	353	122	662	194
G50A1L45	547	570	1319	957
A6140L72	145	383	425	726
A6140L54	1020	966	1924	2071
A6140L32	591	302	1562	652
A6140L75	244	327	429	733
G50A2L35	874	1037	1837	1505
A6140L108	574	351	1622	775
A6140L100	205	130	506	283
A6140L1	591	1038	1696	1923
G50A2L46	1006	1498	1912	2298
A6140L57	722	443	1293	680
A6140L47	728	223	1596	501
A6140L6	381	484	764	842
A6140L53	619	744	1314	1468
A6140L30	585	423	1202	899
A6140L85	363	432	598	805
A6140L36	651	863	1201	1472
A6140L114	533	949	1617	1493
A6140L24	600	355	770	522
A6140L41	401	570	1180	924
A6140L27	169	832	344	1338
A6140L90	344	349	697	637

A6140L71	755	325	1028	584
A6140L74	518	203	1015	378
A6140L92	921	873	2028	1501
A6140L101	710	768	1681	1653
A6140L83	857	447	1248	697
G50A2L43	1411	1498	3150	2451
A6140L52	851	698	1823	1057
A6140L55	555	408	746	496
A6140L86	442	477	801	807
G50A2L34	1057	1183	2294	1891
A6140L23	390	630	568	869
A6140L21	635	827	1382	1487
G50A1L41	1116	1150	2263	1644
G50A2L45	789	1775	1754	2320
A6140L64	156	139	312	252
G50A2L37	1390	2187	3056	2792
A6140L25	424	850	866	1462
G50A2L39	816	1669	1608	2255
A6140L35	305	463	520	667
A6140L99	845	630	1562	1070
A6140L38	740	686	1637	1024
A6140L87	561	848	1015	1363
G50A2L23	921	524	1636	921
A6140L42	733	1035	951	1600
A6140L185	545	468	1202	1000
A6140L15	239	472	585	823
G50A2L44	1109	1741	1930	2468
G50A4L16	840	625	1327	937
A6140L70	113	13	353	38
A6140L77	467	422	1120	804
A6140L9	599	706	1780	1249
A6140L8	786	1063	1557	1837
A6140L80	856	1363	1333	1897
G50A1L9	771	990	1653	1698
G50A1L44	487	447	842	840
G50A1L50	139	337	368	862
A6140L95	127	92	390	206

A6140L192	172	139	526	330
A6140L16	275	562	450	846
G50A2L38	1125	1751	2963	2650
G50A2L32	901	2067	1439	2430
A6140L62	521	484	1065	1055
A6140L96	733	711	1746	1335
G50A1L47	1400	1594	2977	2262
G50A2L41	895	1410	2011	2248
A6140L103	1156	1601	1763	2439
A6140L102	1120	1389	1689	1860
G50A2L33	881	1244	1639	1441
A6140L84	845	948	1584	1665
G50A2L31	1086	1160	1699	1646
A6140L34	830	1720	1458	2279
A6140L68	299	345	824	675
A6140L61	854	655	1588	1099
A6140L26	532	1048	1120	1698
A6140L4	654	907	1559	1809
A6140L91	614	699	1335	1170
A6140L116	1028	998	1766	1358
Total	252469	288303	494290	477452

Appendix 3: Univariate elasticities of selection on all behavioural features.

Linear (B_μ) and quadratic (Γ_μ) elasticities of selection on family means were calculated separately as skewness in behaviour data leads to covariance between linear and quadratic coefficients in the same equation. More details lie in the introduction of this thesis.

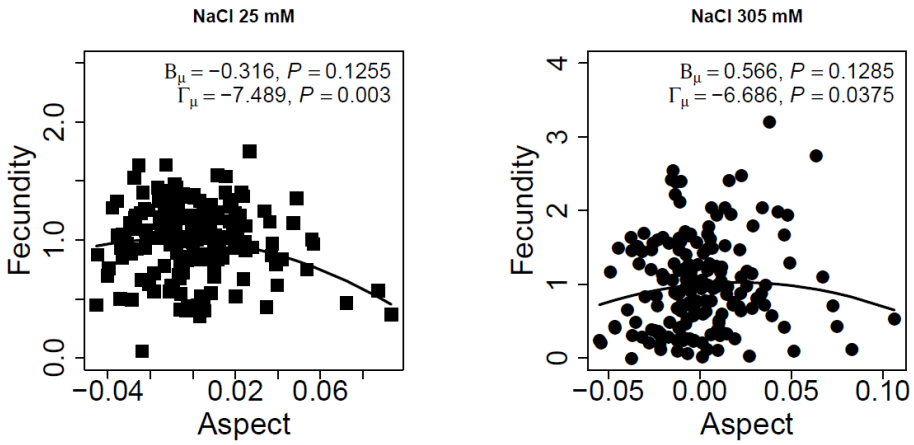
P values were calculated using non-parametric null distributions of selection gradients based on 2,000 replicate permutations of fecundity (our fitness proxy) along behavioural values. For each permutation, regressions were fitted using Equations 1.3 and 1.4; the quadratic coefficient was taken from the first equation and the linear coefficient

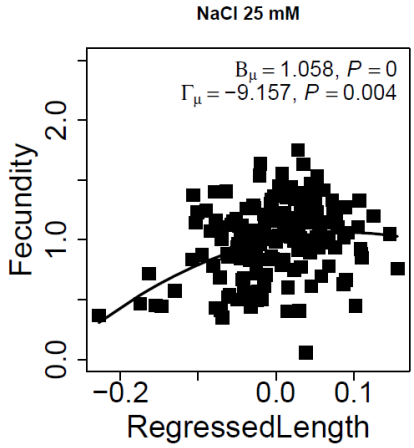
was taken from the second. This process was applied to the 2,000 replicates in order to make two null distributions, one for the quadratic gradients and another for the linear gradients. The selection gradients obtained from the original data were compared with these null distributions. If they were negative, *p* values were defined as the fraction of permuted datasets that had a more negative gradient than the original. If the original coefficients were positive, *p* values were calculated as the fraction of permuted datasets that lead to higher gradients than the original. *P* values equal to zero mean that fraction is zero and that, in a theoretical distribution, such a fraction would be lower than 1/2000.

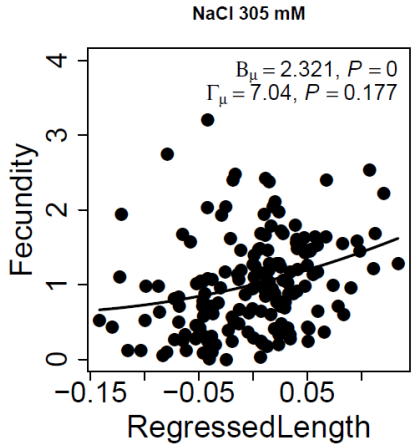
Appendix 3.1: Univariate selection coefficients on behaviour centrality.

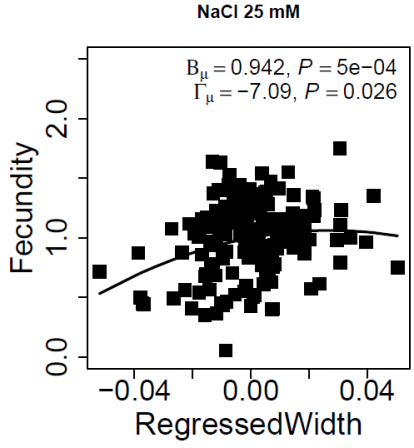
Here are both the univariate selection elasticities and the correspondent univariate selection gradients on behaviour centrality (median), which were used to obtain the elasticities of selection.

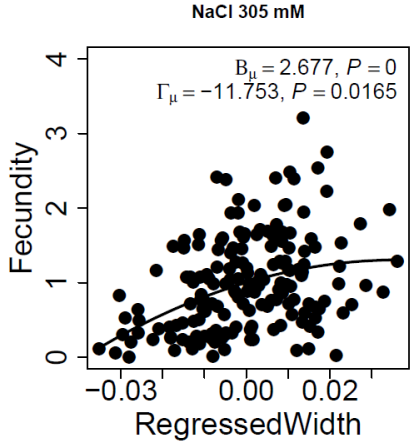
Appendix 3.1.1: Univariate elasticities of selection on behaviour median

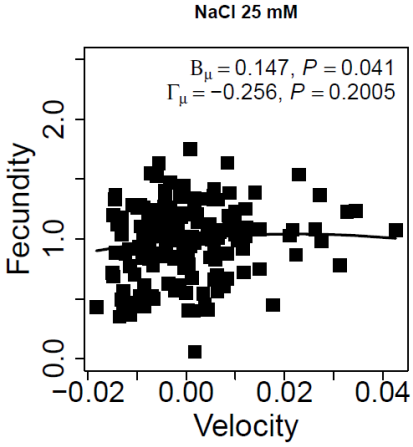


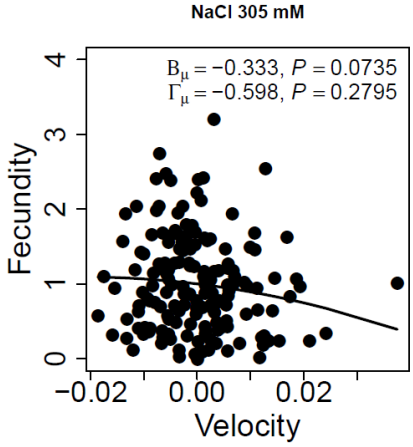

NaCl 25 mM
$B_\mu = 1.058$, $P = 0$
$\Gamma_\mu = -9.157$, $P = 0.004$
Fecundity
RegressedLength

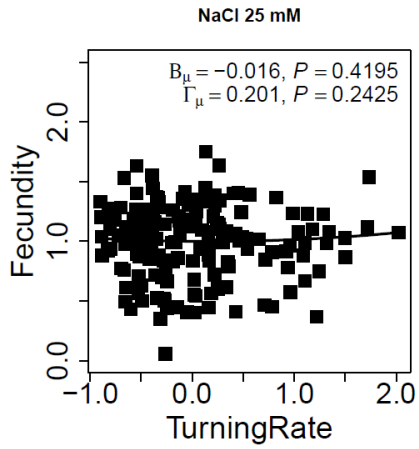

NaCl 305 mM
$B_\mu = 2.321$, $P = 0$
$\Gamma_\mu = 7.04$, $P = 0.177$
Fecundity
RegressedLength

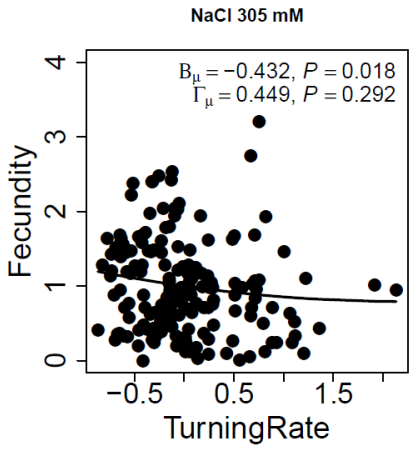

NaCl 25 mM
$B_\mu = 0.942$, $P = 5e{-}04$
$\Gamma_\mu = -7.09$, $P = 0.026$
Fecundity
RegressedWidth

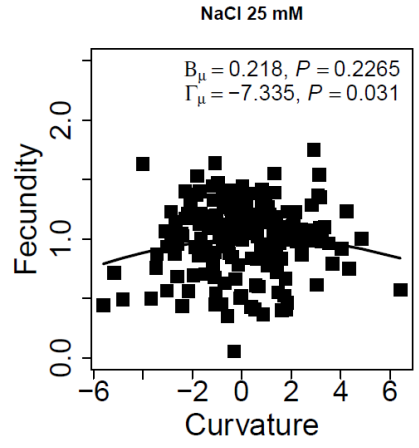

NaCl 305 mM
$B_\mu = 2.677$, $P = 0$
$\Gamma_\mu = -11.753$, $P = 0.0165$
Fecundity
RegressedWidth

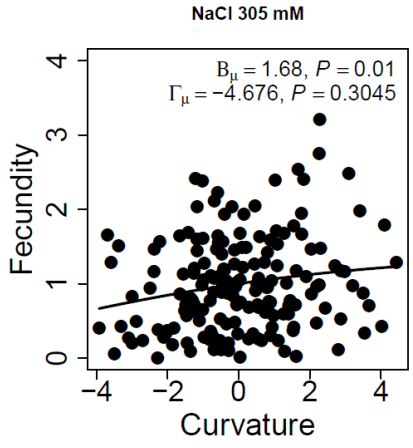

NaCl 25 mM
$B_\mu = 0.147$, $P = 0.041$
$\Gamma_\mu = -0.256$, $P = 0.2005$
Fecundity
Velocity

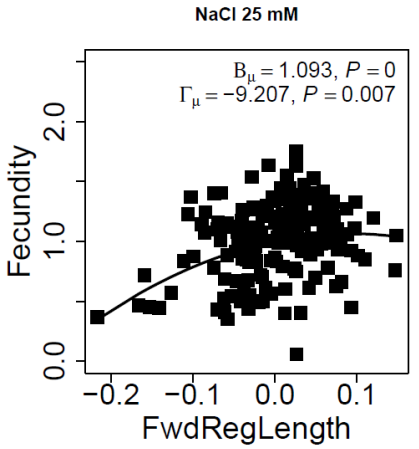

NaCl 305 mM
$B_\mu = -0.333$, $P = 0.0735$
$\Gamma_\mu = -0.598$, $P = 0.2795$
Fecundity
Velocity

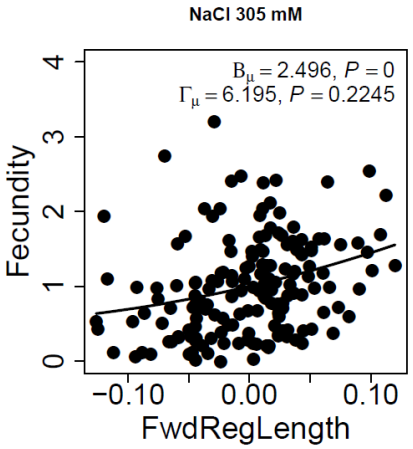

NaCl 25 mM
$B_\mu = -0.016$, $P = 0.4195$
$\Gamma_\mu = 0.201$, $P = 0.2425$
Fecundity
TurningRate

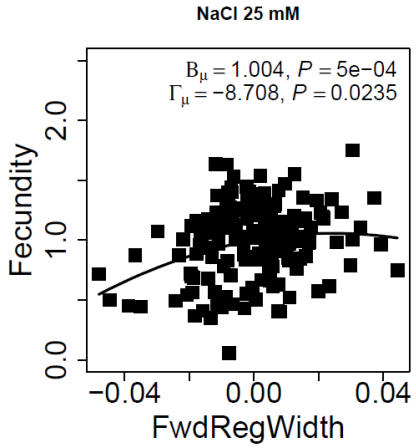

NaCl 305 mM
$B_\mu = -0.432$, $P = 0.018$
$\Gamma_\mu = 0.449$, $P = 0.292$
Fecundity
TurningRate

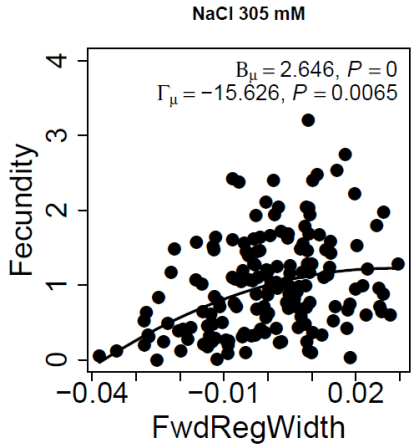

NaCl 25 mM
$B_\mu = 0.218$, $P = 0.2265$
$\Gamma_\mu = -7.335$, $P = 0.031$
Fecundity
Curvature

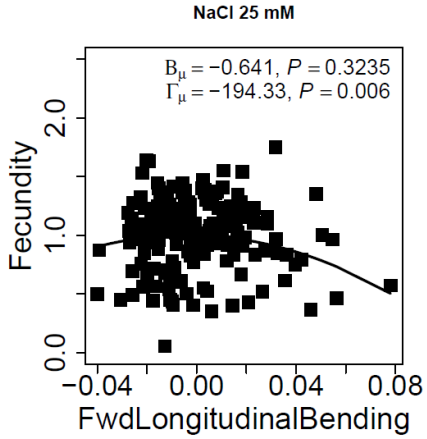

NaCl 305 mM
$B_\mu = 1.68$, $P = 0.01$
$\Gamma_\mu = -4.676$, $P = 0.3045$
Fecundity
Curvature

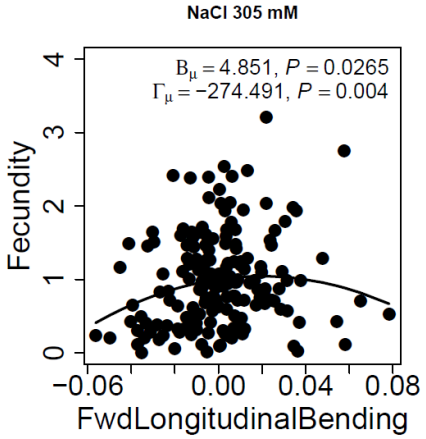

NaCl 25 mM
$B_\mu = 1.093$, $P = 0$
$\Gamma_\mu = -9.207$, $P = 0.007$
Fecundity
FwdRegLength

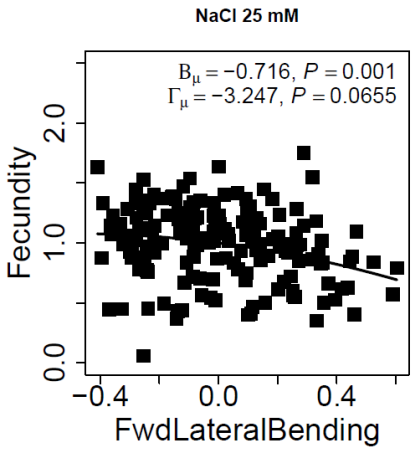

NaCl 305 mM
$B_\mu = 2.496$, $P = 0$
$\Gamma_\mu = 6.195$, $P = 0.2245$
Fecundity
FwdRegLength

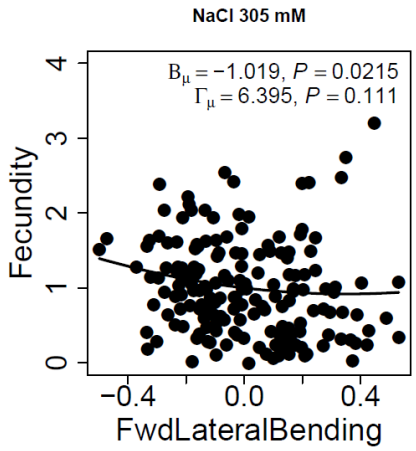

NaCl 25 mM
$B_\mu = 1.004$, $P = 5e{-}04$
$\Gamma_\mu = -8.708$, $P = 0.0235$
Fecundity
FwdRegWidth

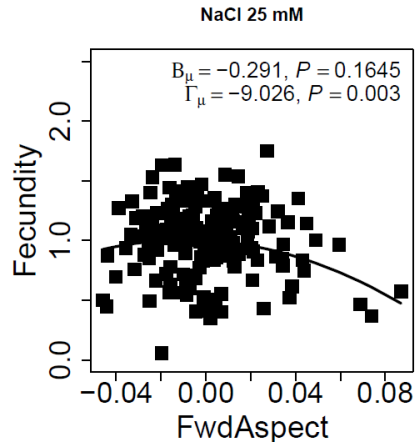

NaCl 305 mM
$B_\mu = 2.646$, $P = 0$
$\Gamma_\mu = -15.626$, $P = 0.0065$
Fecundity
FwdRegWidth

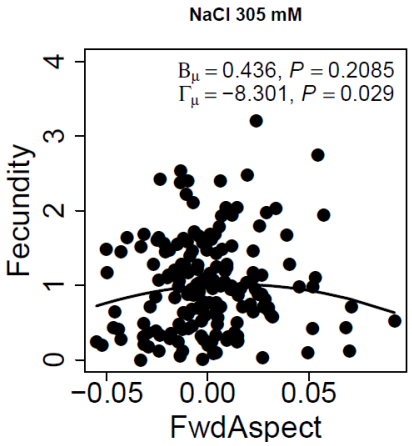

NaCl 25 mM
$B_\mu = -0.641$, $P = 0.3235$
$\Gamma_\mu = -194.33$, $P = 0.006$
Fecundity
FwdLongitudinalBending

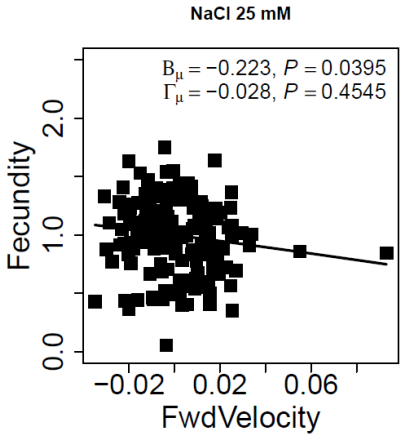

NaCl 305 mM
$B_\mu = 4.851$, $P = 0.0265$
$\Gamma_\mu = -274.491$, $P = 0.004$
Fecundity
FwdLongitudinalBending

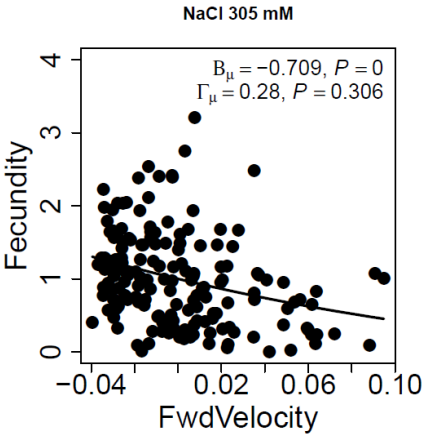

NaCl 25 mM
$B_\mu = -0.716$, $P = 0.001$
$\Gamma_\mu = -3.247$, $P = 0.0655$
Fecundity
FwdLateralBending

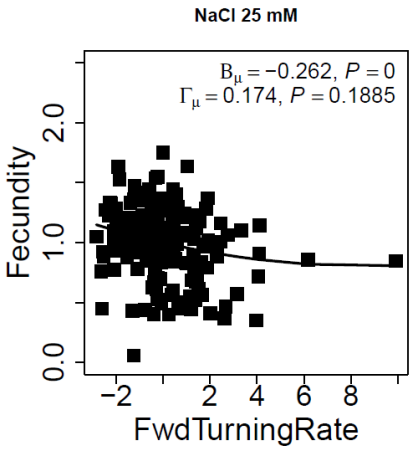

NaCl 305 mM
$B_\mu = -1.019$, $P = 0.0215$
$\Gamma_\mu = 6.395$, $P = 0.111$
Fecundity
FwdLateralBending

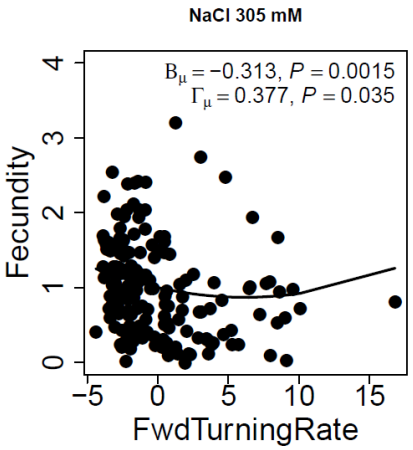

NaCl 25 mM
$B_\mu = -0.291$, $P = 0.1645$
$\Gamma_\mu = -9.026$, $P = 0.003$
Fecundity
FwdAspect

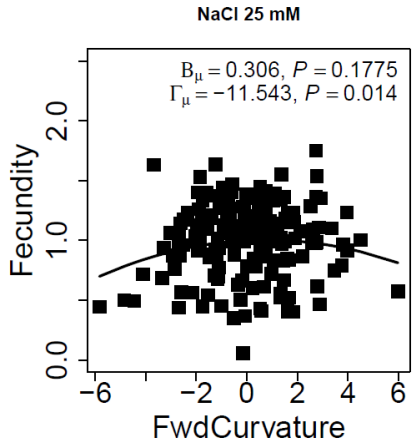

NaCl 305 mM
$B_\mu = 0.436$, $P = 0.2085$
$\Gamma_\mu = -8.301$, $P = 0.029$
Fecundity
FwdAspect

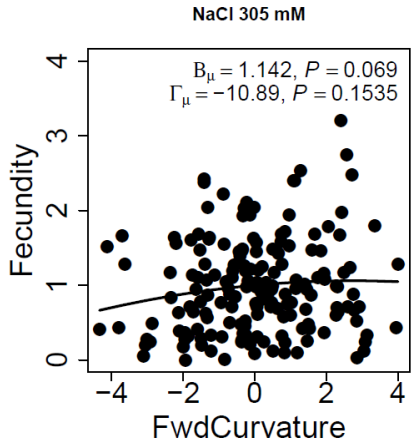

NaCl 25 mM
$B_\mu = -0.223$, $P = 0.0395$
$\Gamma_\mu = -0.028$, $P = 0.4545$
Fecundity
FwdVelocity

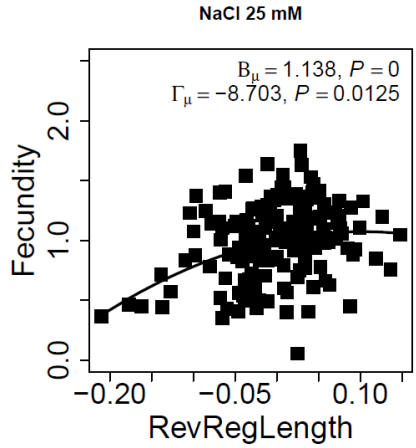

NaCl 305 mM
$B_\mu = -0.709$, $P = 0$
$\Gamma_\mu = 0.28$, $P = 0.306$
Fecundity
FwdVelocity

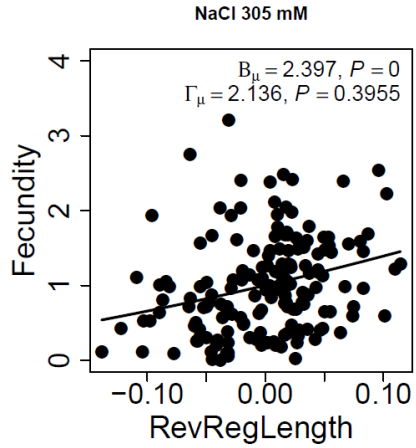

NaCl 25 mM
$B_\mu = -0.262$, $P = 0$
$\Gamma_\mu = 0.174$, $P = 0.1885$
Fecundity
FwdTurningRate

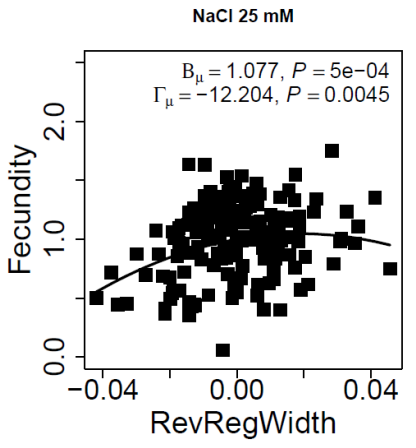

NaCl 305 mM
$B_\mu = -0.313$, $P = 0.0015$
$\Gamma_\mu = 0.377$, $P = 0.035$
Fecundity
FwdTurningRate

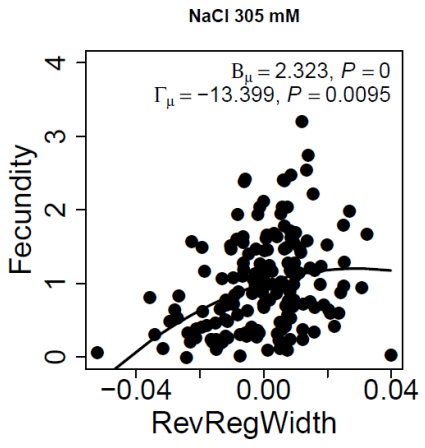

NaCl 25 mM
$B_\mu = 0.306$, $P = 0.1775$
$\Gamma_\mu = -11.543$, $P = 0.014$
Fecundity
FwdCurvature

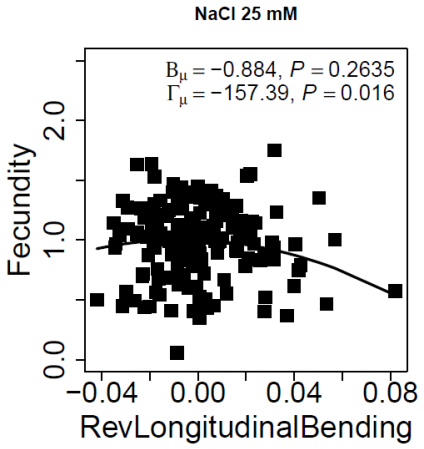

NaCl 305 mM
$B_\mu = 1.142$, $P = 0.069$
$\Gamma_\mu = -10.89$, $P = 0.1535$
Fecundity
FwdCurvature

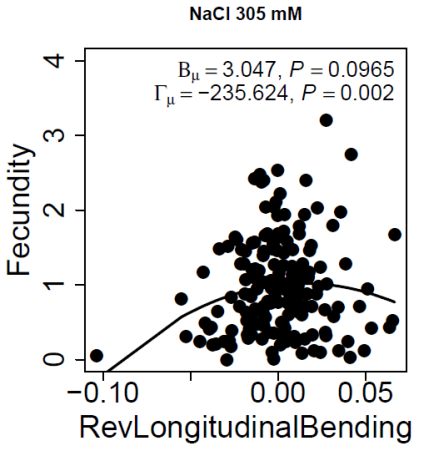

NaCl 25 mM
$B_\mu = 1.138$, $P = 0$
$\Gamma_\mu = -8.703$, $P = 0.0125$
Fecundity
RevRegLength

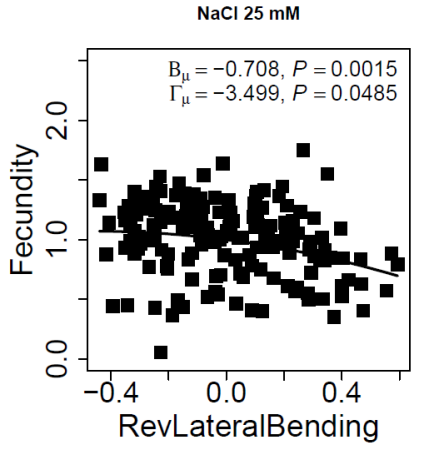

NaCl 305 mM
$B_\mu = 2.397$, $P = 0$
$\Gamma_\mu = 2.136$, $P = 0.3955$
Fecundity
RevRegLength

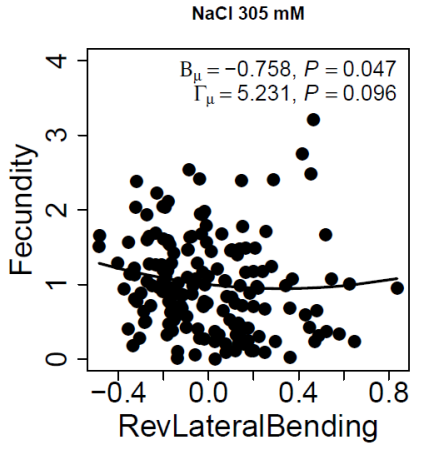

NaCl 25 mM
$B_\mu = 1.077$, $P = 5e{-}04$
$\Gamma_\mu = -12.204$, $P = 0.0045$
Fecundity
RevRegWidth

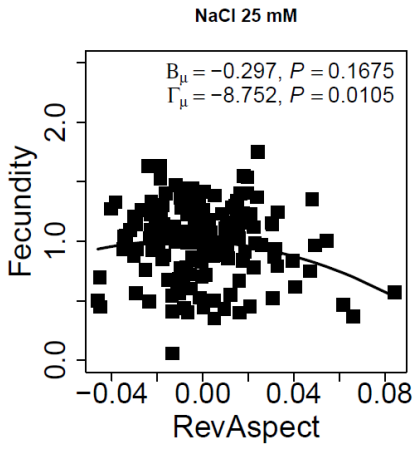

NaCl 305 mM
$B_\mu = 2.323$, $P = 0$
$\Gamma_\mu = -13.399$, $P = 0.0095$
Fecundity
RevRegWidth

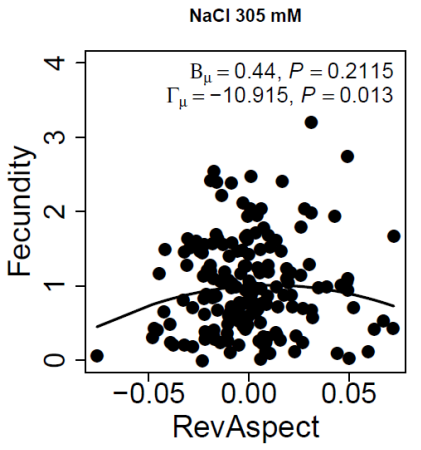

NaCl 25 mM
$B_\mu = -0.884$, $P = 0.2635$
$\Gamma_\mu = -157.39$, $P = 0.016$
Fecundity
RevLongitudinalBending

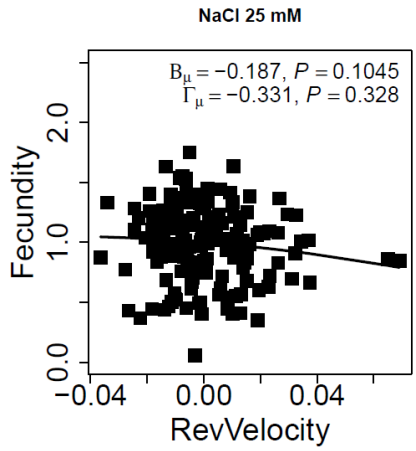

NaCl 305 mM
$B_\mu = 3.047$, $P = 0.0965$
$\Gamma_\mu = -235.624$, $P = 0.002$
Fecundity
RevLongitudinalBending

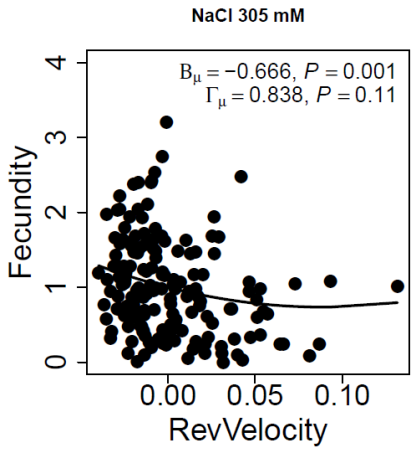

NaCl 25 mM
$B_\mu = -0.708$, $P = 0.0015$
$\Gamma_\mu = -3.499$, $P = 0.0485$
Fecundity
RevLateralBending

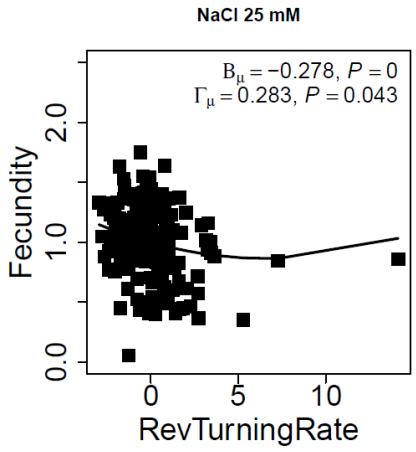

NaCl 305 mM
$B_\mu = -0.758$, $P = 0.047$
$\Gamma_\mu = 5.231$, $P = 0.096$
Fecundity
RevLateralBending

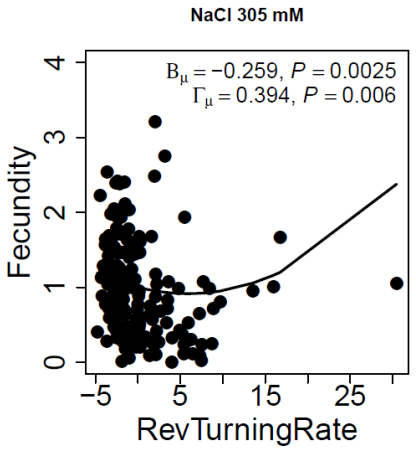

NaCl 25 mM
$B_\mu = -0.297$, $P = 0.1675$
$\Gamma_\mu = -8.752$, $P = 0.0105$
Fecundity
RevAspect

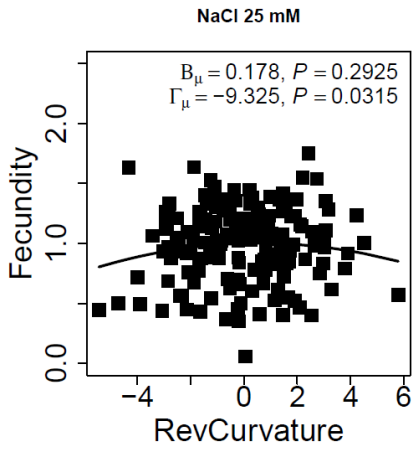

NaCl 305 mM
$B_\mu = 0.44$, $P = 0.2115$
$\Gamma_\mu = -10.915$, $P = 0.013$
Fecundity
RevAspect

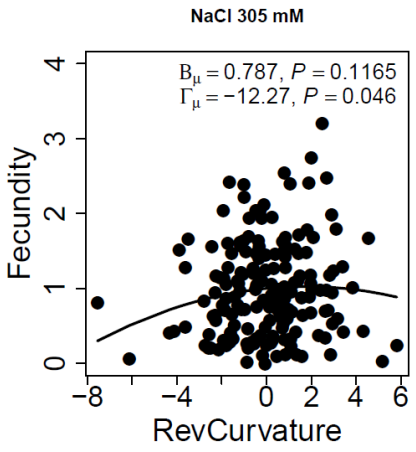

NaCl 25 mM
$B_\mu = -0.187$, $P = 0.1045$
$\Gamma_\mu = -0.331$, $P = 0.328$
Fecundity
RevVelocity

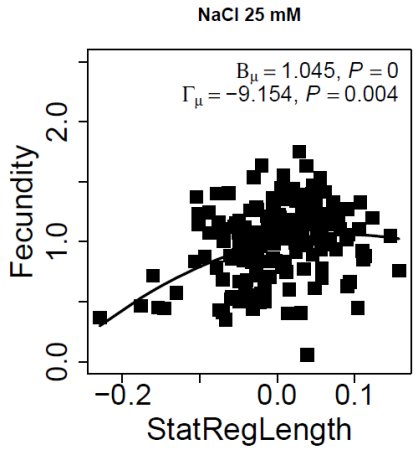

NaCl 305 mM
$B_\mu = -0.666$, $P = 0.001$
$\Gamma_\mu = 0.838$, $P = 0.11$
Fecundity
RevVelocity

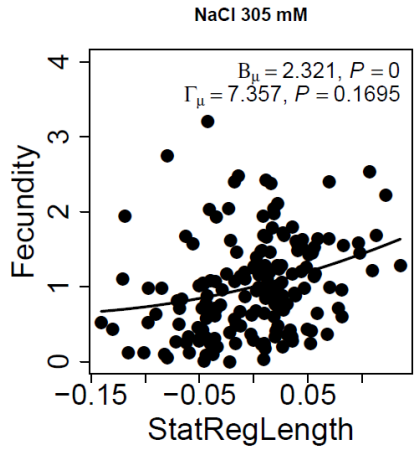

NaCl 25 mM
$B_\mu = -0.278$, $P = 0$
$\Gamma_\mu = 0.283$, $P = 0.043$
Fecundity
RevTurningRate

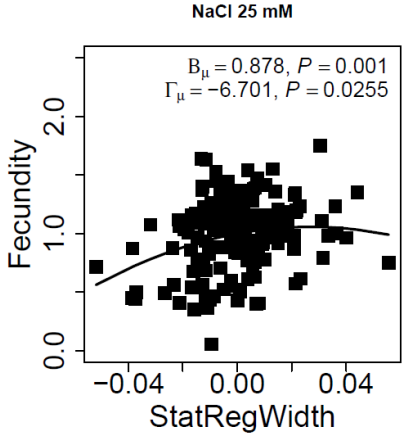

NaCl 305 mM
$B_\mu = -0.259$, $P = 0.0025$
$\Gamma_\mu = 0.394$, $P = 0.006$
Fecundity
RevTurningRate

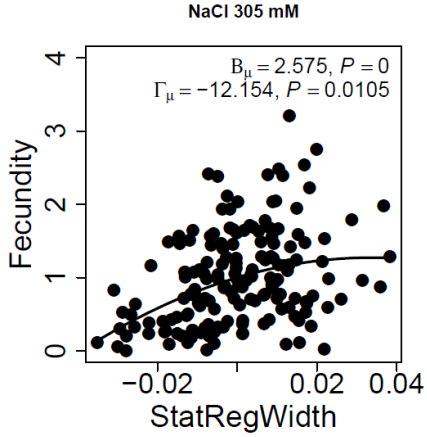

NaCl 25 mM
$B_\mu = 0.178$, $P = 0.2925$
$\Gamma_\mu = -9.325$, $P = 0.0315$
Fecundity
RevCurvature

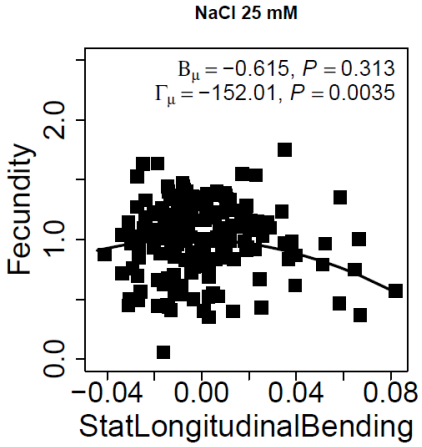

NaCl 305 mM
$B_\mu = 0.787$, $P = 0.1165$
$\Gamma_\mu = -12.27$, $P = 0.046$
Fecundity
RevCurvature

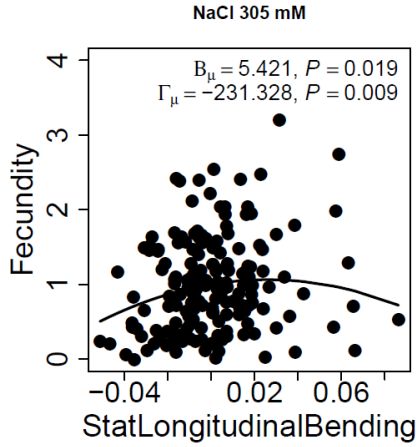

NaCl 25 mM
$B_\mu = 1.045$, $P = 0$
$\Gamma_\mu = -9.154$, $P = 0.004$
Fecundity
StatRegLength

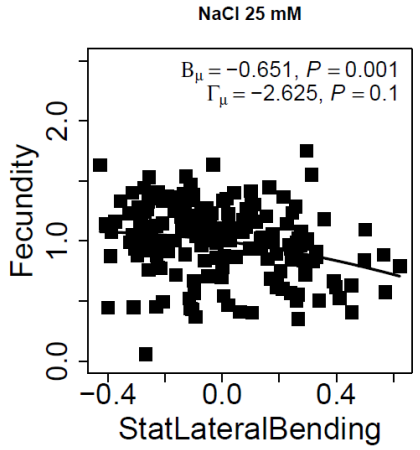

NaCl 305 mM
$B_\mu = 2.321$, $P = 0$
$\Gamma_\mu = 7.357$, $P = 0.1695$
Fecundity
StatRegLength

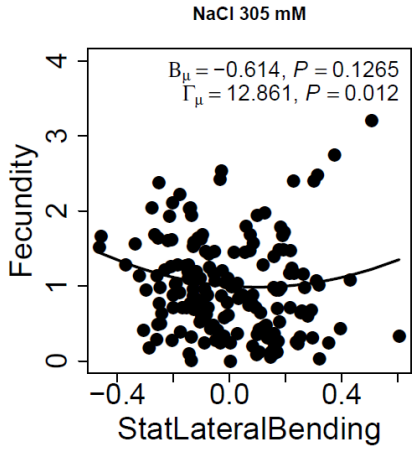

NaCl 25 mM
$B_\mu = 0.878$, $P = 0.001$
$\Gamma_\mu = -6.701$, $P = 0.0255$
Fecundity
StatRegWidth

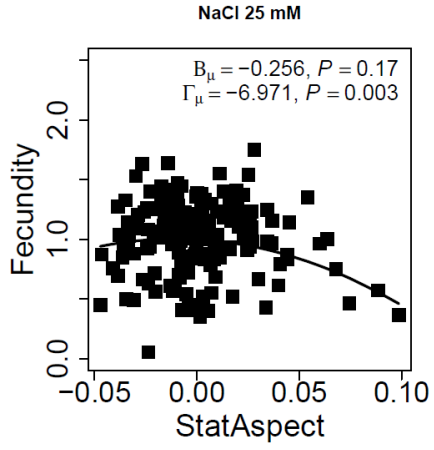

NaCl 305 mM
$B_\mu = 2.575$, $P = 0$
$\Gamma_\mu = -12.154$, $P = 0.0105$
Fecundity
StatRegWidth

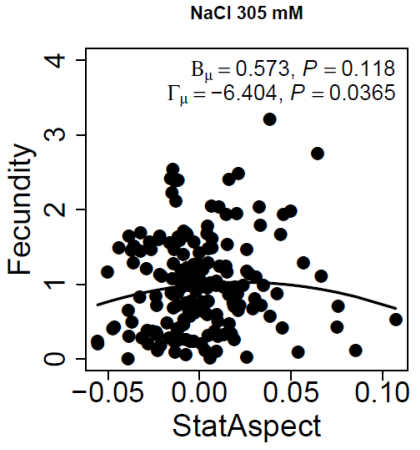

NaCl 25 mM
$B_\mu = -0.615$, $P = 0.313$
$\Gamma_\mu = -152.01$, $P = 0.0035$
Fecundity
StatLongitudinalBending

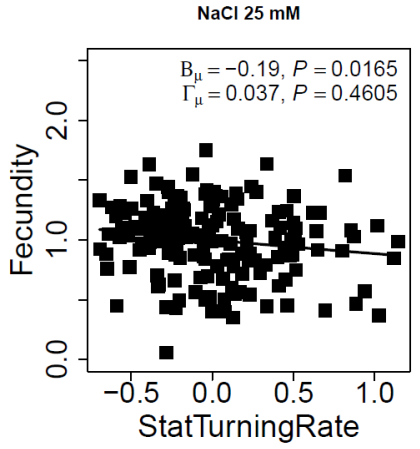

NaCl 305 mM
$B_\mu = 5.421$, $P = 0.019$
$\Gamma_\mu = -231.328$, $P = 0.009$
Fecundity
StatLongitudinalBending

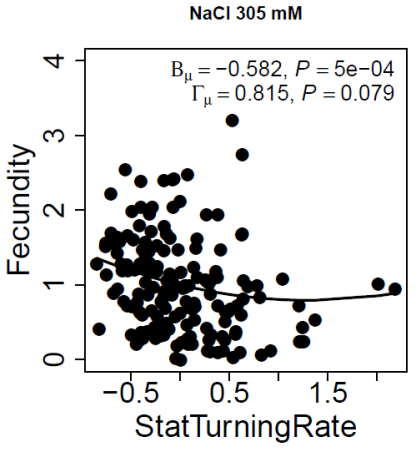

NaCl 25 mM
$B_\mu = -0.651$, $P = 0.001$
$\Gamma_\mu = -2.625$, $P = 0.1$
Fecundity
StatLateralBending

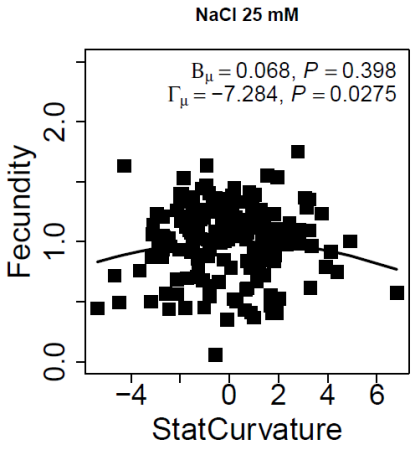

NaCl 305 mM
$B_\mu = -0.614$, $P = 0.1265$
$\Gamma_\mu = 12.861$, $P = 0.012$
Fecundity
StatLateralBending

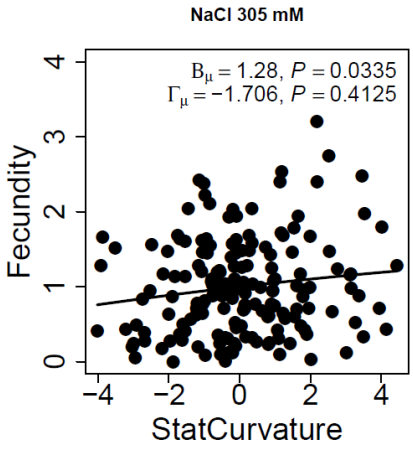

NaCl 25 mM
$B_\mu = -0.256$, $P = 0.17$
$\Gamma_\mu = -6.971$, $P = 0.003$
Fecundity
StatAspect

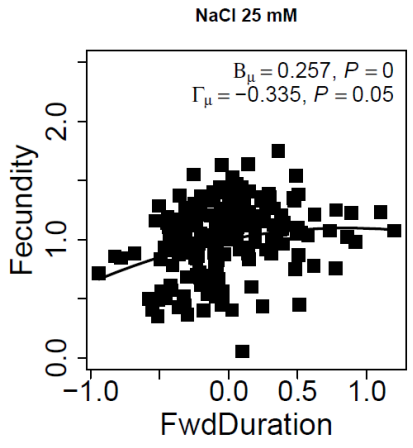

NaCl 305 mM
$B_\mu = 0.573$, $P = 0.118$
$\Gamma_\mu = -6.404$, $P = 0.0365$
Fecundity
StatAspect

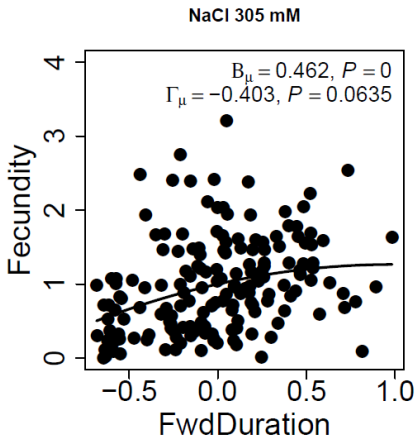

NaCl 25 mM
$B_\mu = -0.19$, $P = 0.0165$
$\Gamma_\mu = 0.037$, $P = 0.4605$
Fecundity
StatTurningRate

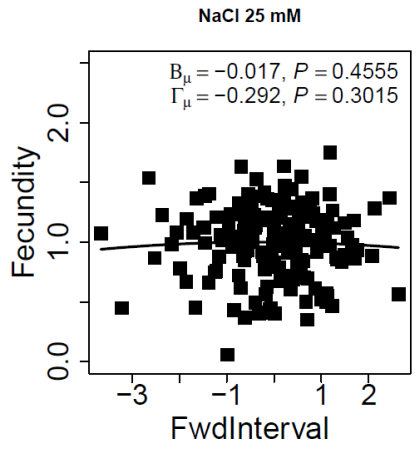

NaCl 305 mM
$B_\mu = -0.582$, $P = 5e{-}04$
$\Gamma_\mu = 0.815$, $P = 0.079$
Fecundity
StatTurningRate

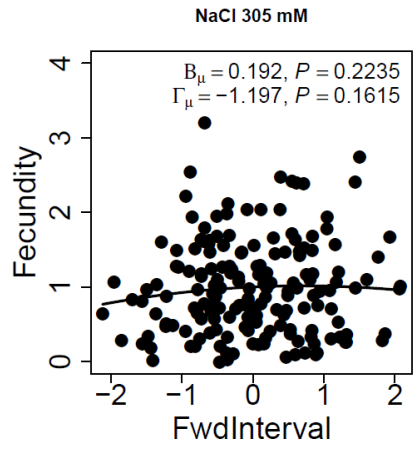

NaCl 25 mM
$B_\mu = 0.068$, $P = 0.398$
$\Gamma_\mu = -7.284$, $P = 0.0275$
Fecundity
StatCurvature

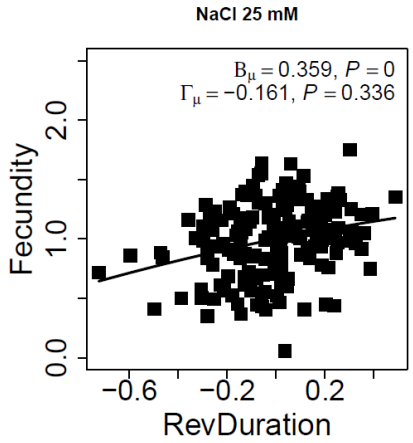

NaCl 305 mM
$B_\mu = 1.28$, $P = 0.0335$
$\Gamma_\mu = -1.706$, $P = 0.4125$
Fecundity
StatCurvature

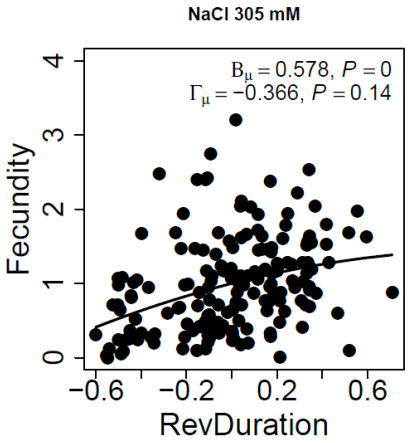

NaCl 25 mM
$B_\mu = 0.257$, $P = 0$
$\Gamma_\mu = -0.335$, $P = 0.05$
Fecundity
FwdDuration

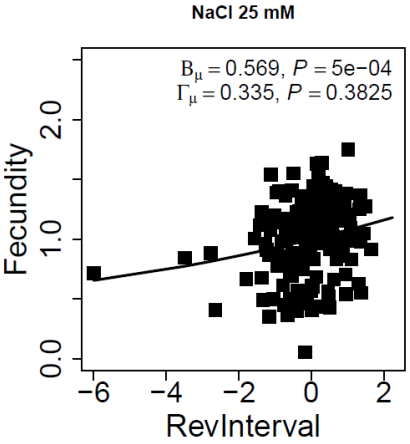

NaCl 305 mM
$B_\mu = 0.462$, $P = 0$
$\Gamma_\mu = -0.403$, $P = 0.0635$
Fecundity
FwdDuration

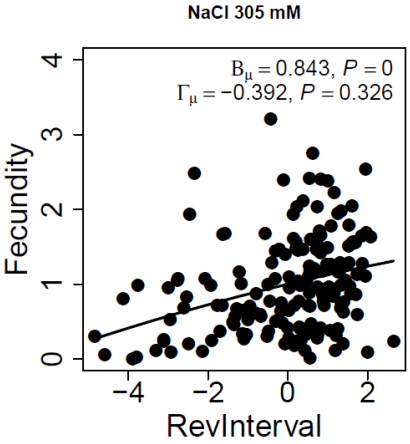

NaCl 25 mM
$B_\mu = -0.017$, $P = 0.4555$
$\Gamma_\mu = -0.292$, $P = 0.3015$
Fecundity
FwdInterval

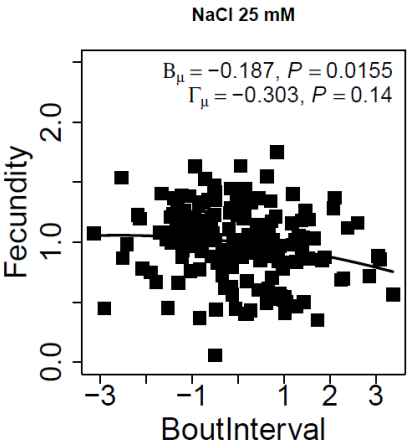

NaCl 305 mM
$B_\mu = 0.192$, $P = 0.2235$
$\Gamma_\mu = -1.197$, $P = 0.1615$
Fecundity
FwdInterval

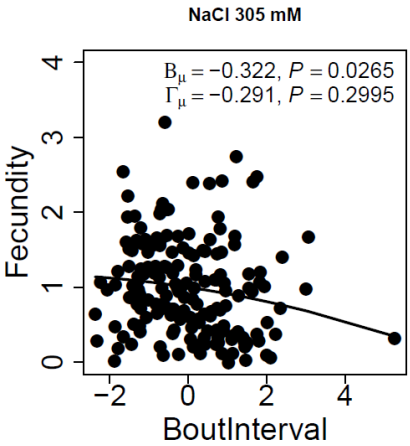

NaCl 25 mM
$B_\mu = 0.359$, $P = 0$
$\Gamma_\mu = -0.161$, $P = 0.336$
Fecundity
RevDuration

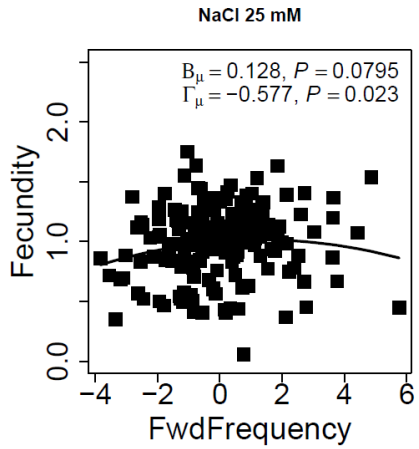

NaCl 305 mM
$B_\mu = 0.578$, $P = 0$
$\Gamma_\mu = -0.366$, $P = 0.14$
Fecundity
RevDuration

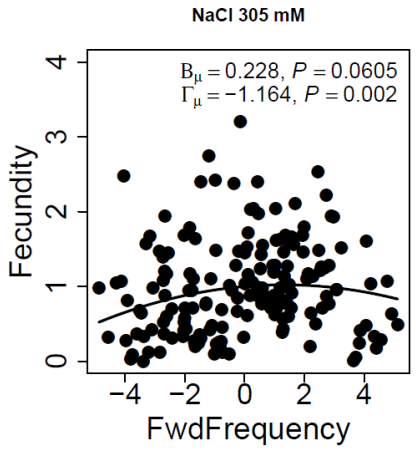

NaCl 25 mM
$B_\mu = 0.569$, $P = 5e{-}04$
$\Gamma_\mu = 0.335$, $P = 0.3825$
Fecundity
RevInterval

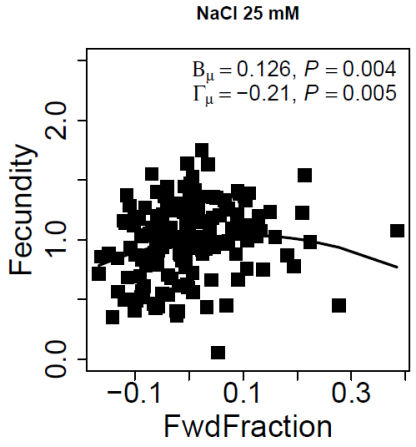

NaCl 305 mM
$B_\mu = 0.843$, $P = 0$
$\Gamma_\mu = -0.392$, $P = 0.326$
Fecundity
RevInterval

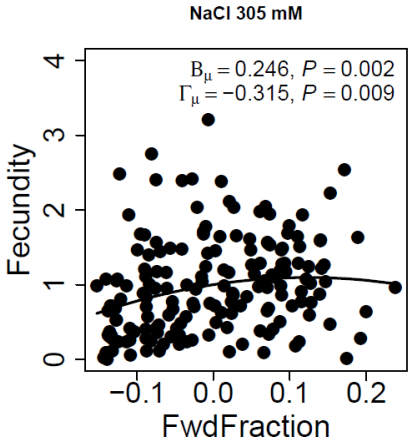

NaCl 25 mM
$B_\mu = -0.187$, $P = 0.0155$
$\Gamma_\mu = -0.303$, $P = 0.14$
Fecundity
BoutInterval

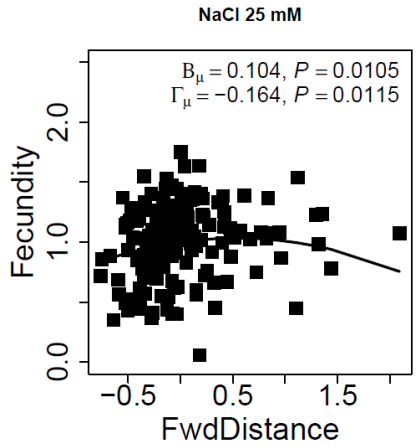

NaCl 305 mM
$B_\mu = -0.322$, $P = 0.0265$
$\Gamma_\mu = -0.291$, $P = 0.2995$
Fecundity
BoutInterval

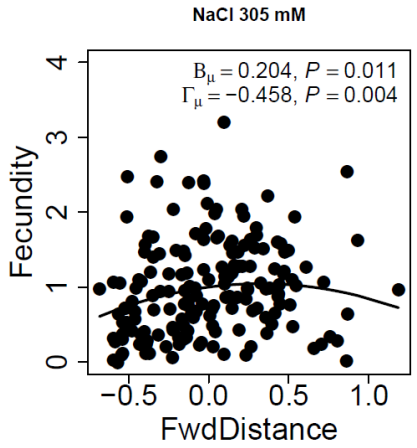

NaCl 25 mM
$B_\mu = 0.128$, $P = 0.0795$
$\Gamma_\mu = -0.577$, $P = 0.023$
Fecundity
FwdFrequency

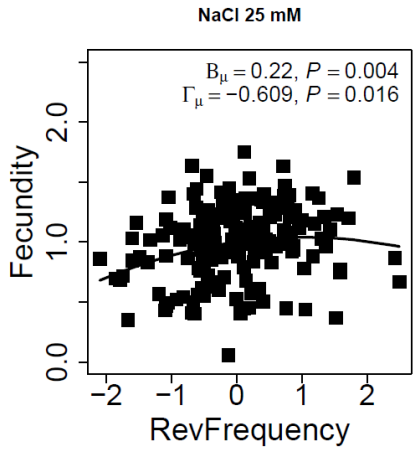

NaCl 305 mM
$B_\mu = 0.228$, $P = 0.0605$
$\Gamma_\mu = -1.164$, $P = 0.002$
Fecundity
FwdFrequency

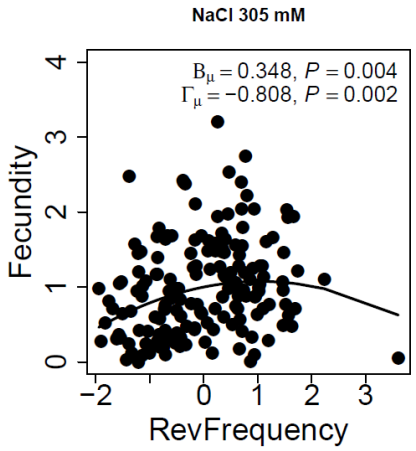

NaCl 25 mM
$B_\mu = 0.126$, $P = 0.004$
$\Gamma_\mu = -0.21$, $P = 0.005$
Fecundity
FwdFraction

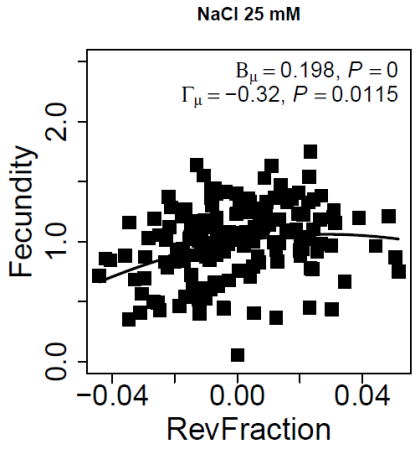

NaCl 305 mM
$B_\mu = 0.246$, $P = 0.002$
$\Gamma_\mu = -0.315$, $P = 0.009$
Fecundity
FwdFraction

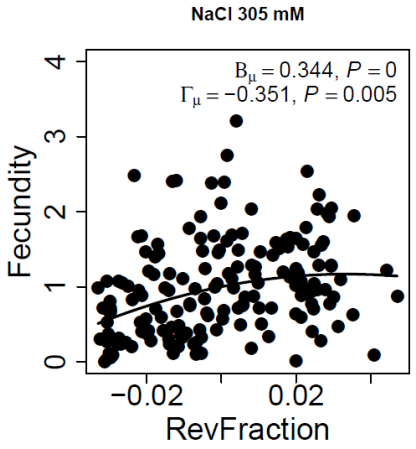

NaCl 25 mM
$B_\mu = 0.104$, $P = 0.0105$
$\Gamma_\mu = -0.164$, $P = 0.0115$
Fecundity
FwdDistance

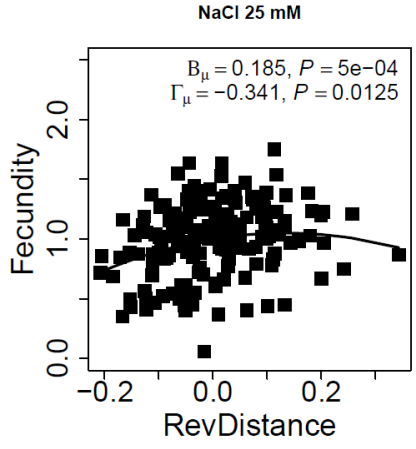

NaCl 305 mM
$B_\mu = 0.204$, $P = 0.011$
$\Gamma_\mu = -0.458$, $P = 0.004$
Fecundity
FwdDistance

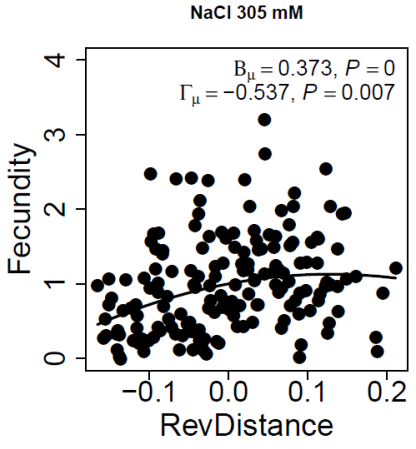

NaCl 25 mM
$B_\mu = 0.22$, $P = 0.004$
$\Gamma_\mu = -0.609$, $P = 0.016$
Fecundity
RevFrequency

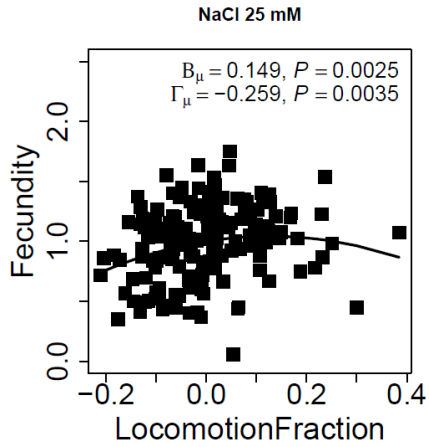

NaCl 305 mM
$B_\mu = 0.348$, $P = 0.004$
$\Gamma_\mu = -0.808$, $P = 0.002$
Fecundity
RevFrequency

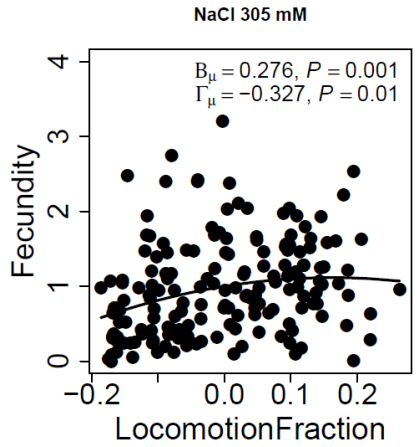

NaCl 25 mM
$B_\mu = 0.198$, $P = 0$
$\Gamma_\mu = -0.32$, $P = 0.0115$
Fecundity
RevFraction

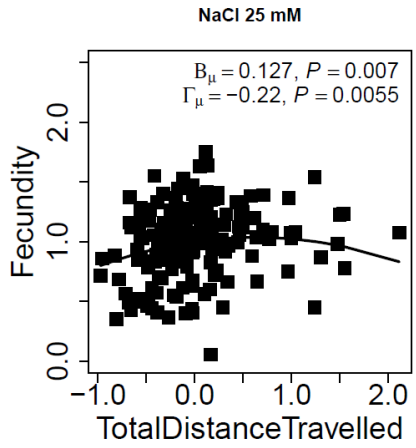

NaCl 305 mM
$B_\mu = 0.344$, $P = 0$
$\Gamma_\mu = -0.351$, $P = 0.005$
Fecundity
RevFraction

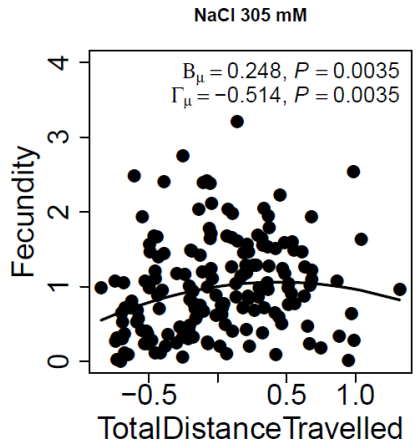

NaCl 25 mM
$B_\mu = 0.185$, $P = 5e{-}04$
$\Gamma_\mu = -0.341$, $P = 0.0125$
Fecundity
RevDistance

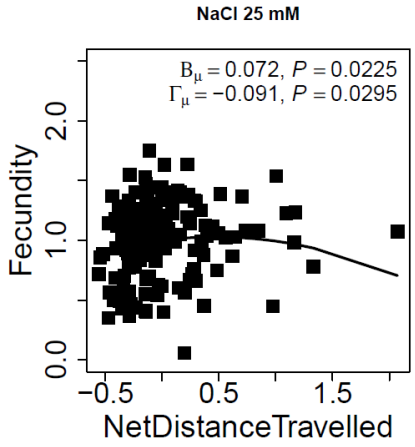

NaCl 305 mM
$B_\mu = 0.373$, $P = 0$
$\Gamma_\mu = -0.537$, $P = 0.007$
Fecundity
RevDistance

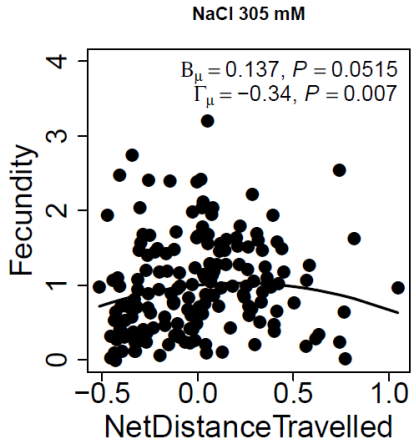

NaCl 25 mM
$B_\mu = 0.149$, $P = 0.0025$
$\Gamma_\mu = -0.259$, $P = 0.0035$
Fecundity
LocomotionFraction

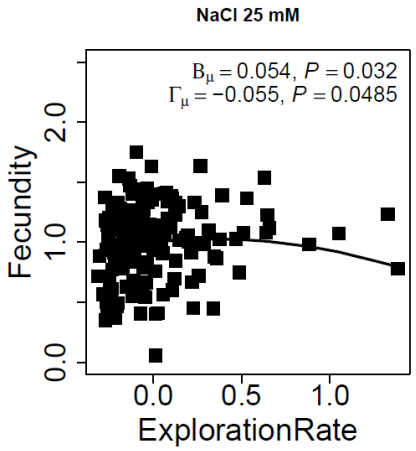

NaCl 305 mM
$B_\mu = 0.276$, $P = 0.001$
$\Gamma_\mu = -0.327$, $P = 0.01$
Fecundity
LocomotionFraction

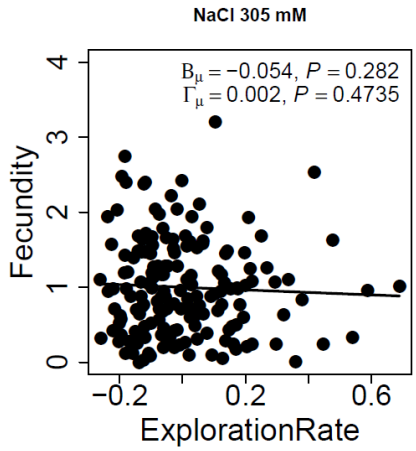

NaCl 25 mM
$B_\mu = 0.127$, $P = 0.007$
$\Gamma_\mu = -0.22$, $P = 0.0055$
Fecundity
TotalDistanceTravelled

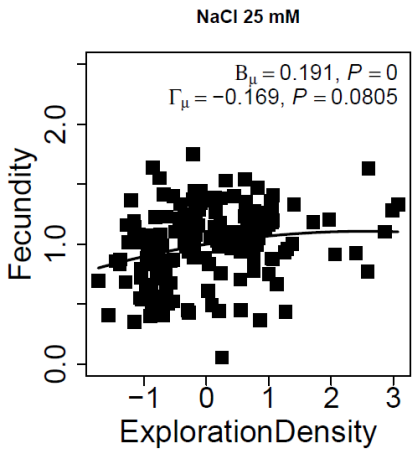

NaCl 305 mM
$B_\mu = 0.248$, $P = 0.0035$
$\Gamma_\mu = -0.514$, $P = 0.0035$
Fecundity
TotalDistanceTravelled

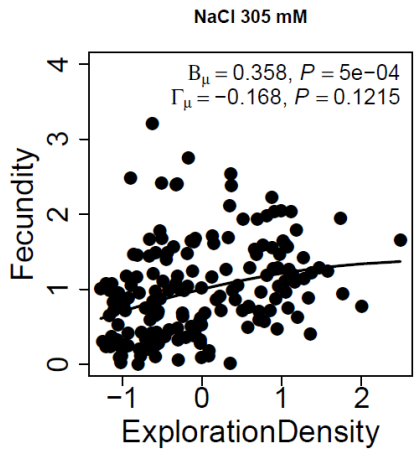

NaCl 25 mM
$B_\mu = 0.072$, $P = 0.0225$
$\Gamma_\mu = -0.091$, $P = 0.0295$
Fecundity
NetDistanceTravelled

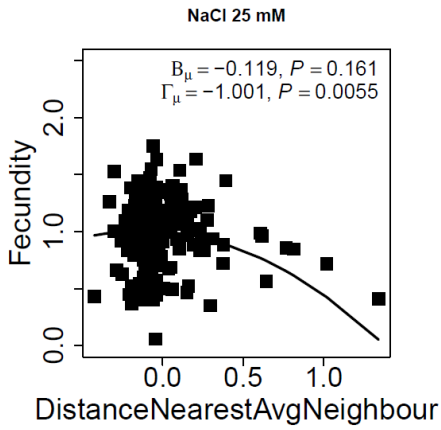

NaCl 305 mM
$B_\mu = 0.137$, $P = 0.0515$
$\Gamma_\mu = -0.34$, $P = 0.007$
Fecundity
NetDistanceTravelled

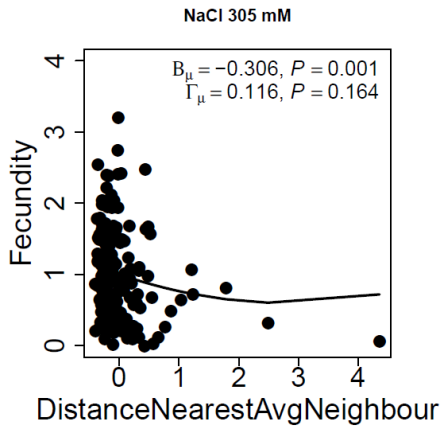

NaCl 25 mM
$B_\mu = 0.054$, $P = 0.032$
$\Gamma_\mu = -0.055$, $P = 0.0485$
Fecundity
ExplorationRate

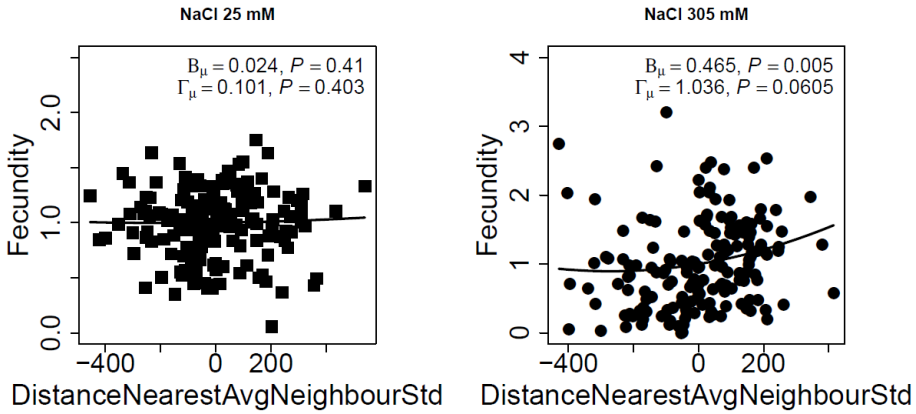

NaCl 305 mM
$B_\mu = -0.054$, $P = 0.282$
$\Gamma_\mu = 0.002$, $P = 0.4735$
Fecundity
ExplorationRate


NaCl 25 mM
$B_\mu = 0.191$, $P = 0$
$\Gamma_\mu = -0.169$, $P = 0.0805$
Fecundity
ExplorationDensity


NaCl 305 mM
$B_\mu = 0.358$, $P = 5e{-}04$
$\Gamma_\mu = -0.168$, $P = 0.1215$
Fecundity
ExplorationDensity


NaCl 25 mM
$B_\mu = -0.119$, $P = 0.161$
$\Gamma_\mu = -1.001$, $P = 0.0055$
Fecundity
DistanceNearestAvgNeighbour


NaCl 305 mM
$B_\mu = -0.306$, $P = 0.001$
$\Gamma_\mu = 0.116$, $P = 0.164$
Fecundity
DistanceNearestAvgNeighbour



Appendix 3.1.2: Univariate selection estimates for behaviour centrality features.

In the following tables I present the estimates of selection gradients (B) and selection curvature (Γ) on behaviour centrality (median) corresponding to the elasticities of selection shown above. Subscripts specify the environment, defined by NaCl concentration. Asterisks highlight the statistical significant estimates under a significance level of 0.05 and the *P* values are the same ones that are on the respective plots above.

Feature	B_{25}	Standard error	*P*
Aspect	−1.15580901	0.986825458	0.1255
RegressedLength	1.35009719	0.358710507	0*
RegressedWidth	4.600019701	1.485371593	0.0005*
Velocity	3.806752206	2.205547768	0.041*
TurningRate	−0.007439614	0.038173278	0.4195
Curvature	0.008206798	0.011741823	0.2265
FwdRegLength	1.39306220	0.378233386	0*
FwdRegWidth	4.758322061	1.508822954	0.0005*
FwdLongitudinalBending	−0.559792087	1.197484967	0.3235
FwdLateralBending	−0.31726655	0.097731862	0.001*
FwdAspect	−1.038695201	1.035745938	0.1645
FwdVelocity	−2.525016267	1.379714203	0.0395*
FwdTurningRate	−0.045321927	0.013065776	0*
FwdCurvature	0.010668506	0.012130474	0.1775

RevRegLength	1.4599683	0.38469937	0*
RevRegWidth	5.040575108	1.507359397	0.0005*
RevLongitudinalBending	−0.766876055	1.187574922	0.2635
RevLateralBending	−0.31033211	0.094880187	0.0015*
RevAspect	−1.033925476	1.067362663	0.1675
RevVelocity	−1.87306147	1.460277156	0.1045
RevTurningRate	−0.0408257	0.011807432	0*
RevCurvature	0.006126218	0.011886745	0.2925
StatRegLength	1.33786984	0.359548503	0*
StatRegWidth	4.288966289	1.438946941	0.001*
StatLongitudinalBending	−0.540976603	1.072622881	0.313
StatLateralBending	−0.29932092	0.098658984	0.001*
StatAspect	−0.929236613	0.934185358	0.17
StatTurningRate	−0.12146611	0.057368323	0.0165*
StatCurvature	0.002602415	0.011821665	0.398
FwdDuration	0.23016274	0.061409414	0*
FwdInterval	−0.003201243	0.022048956	0.4555
RevDuration	0.41330073	0.110688327	0*
RevInterval	0.074676971	0.024367814	0.0005*
BoutInterval	−0.044374137	0.019113161	0.0155*
FwdFrequency	0.021031143	0.014116505	0.0795
FwdFraction	0.720629389	0.26350123	0.004*
FwdDistance	0.12310970	0.05119642	0.0105*
RevFrequency	0.075953988	0.027666441	0.004*
RevFraction	4.300059339	1.187563806	0*
RevDistance	0.748326181	0.234674256	0.0005*
LocomotionFraction	0.6729418	0.2236603	0.0025*
TotalDistanceTravelled	0.11591591	0.043715817	0.007*
NetDistanceTravelled	0.12089558	0.060175835	0.0225*
ExplorationRate	0.15163431	0.083795465	0.032*
ExplorationDensity	0.085183364	0.023823741	0*
DistanceNearestAvgNeighbour	−0.111535796	0.101639793	0.161
DistanceNearestAvgNeighbourStd	3.31×10^{-05}	0.000134242	0.41

Feature	**B_{305}**	**Standard error**	***P***
Aspect	2.233407986	1.867493581	0.1285
RegressedLength	3.53027871	0.900090356	0*
RegressedWidth	16.78588389	3.289331453	0*
Velocity	−8.629694579	5.773947132	0.0735
TurningRate	−0.18837206	0.090007259	0.018*
Curvature	0.069344806	0.028147581	0.01*
FwdRegLength	3.77404176	0.950703928	0*
FwdRegWidth	16.02222235	3.412230193	0*
FwdLongitudinalBending	4.33589318	2.215336476	0.0265*
FwdLateralBending	−0.46106233	0.225895636	0.0215*
FwdAspect	1.672953416	1.936351922	0.2085

FwdVelocity	−7.107890908	1.603468846	0*
FwdTurningRate	−0.042265678	0.013307264	0.0015*
FwdCurvature	0.043404828	0.028699764	0.069
RevRegLength	3.67344748	0.977707139	0*
RevRegWidth	13.735772	3.30763954	0*
RevLongitudinalBending	2.6961925	2.078202971	0.0965
RevLateralBending	−0.335770508	0.195886535	0.047*
RevAspect	1.623917334	1.959424059	0.2115
RevVelocity	−5.919887666	1.637042791	0.001*
RevTurningRate	−0.028704929	0.010778469	0.0025*
RevCurvature	0.029156849	0.024368315	0.1165
StatRegLength	3.54441767	0.910845017	0*
StatRegWidth	16.17038713	3.254661561	0*
StatLongitudinalBending	4.886498818	2.160415395	0.019*
StatLateralBending	−0.290647195	0.249754777	0.1265
StatAspect	2.24449341	1.813284378	0.118
StatTurningRate	−0.318656493	0.090768853	0.0005*
StatCurvature	0.053877921	0.02802445	0.0335*
FwdDuration	0.53948359	0.12511809	0*
FwdInterval	0.043762291	0.055762731	0.2235
RevDuration	0.779852945	0.163480235	0*
RevInterval	0.12875627	0.030998309	0*
BoutInterval	−0.078937037	0.039448952	0.0265*
FwdFrequency	0.034146113	0.021039242	0.0605
FwdFraction	1.55764658	0.509039814	0.002*
FwdDistance	0.280032786	0.126010554	0.011*
RevFrequency	0.14340789	0.048914896	0.004*
RevFraction	9.987405573	2.293365535	0*
RevDistance	2.00407552	0.527046861	0*
LocomotionFraction	1.43248661	0.425861967	0.001*
TotalDistanceTravelled	0.27152157	0.104791648	0.0035*
NetDistanceTravelled	0.253220364	0.154124649	0.0515
ExplorationRate	−0.176603603	0.283971109	0.282
ExplorationDensity	0.25376574	0.056557914	0.0005*
DistanceNearestAvgNeighbour	−0.28665457	0.09452245	0.001*
DistanceNearestAvgNeighbourStd	0.000769039	0.000298466	0.005*

Feature	Γ_{25}	**Standard error**	***P***
Aspect	−100.4910404	41.81212535	0.003*
RegressedLength	−14.90587204	6.042658862	0.004*
RegressedWidth	−169.1210652	94.32348001	0.026*
Velocity	−171.873778	223.423205	0.2005
TurningRate	0.043087977	0.079351808	0.2425
Curvature	−0.01036847	0.006499346	0.031*
FwdRegLength	−14.94677075	6.621086385	0.007*
FwdRegWidth	−195.5394204	101.4535608	0.0235*

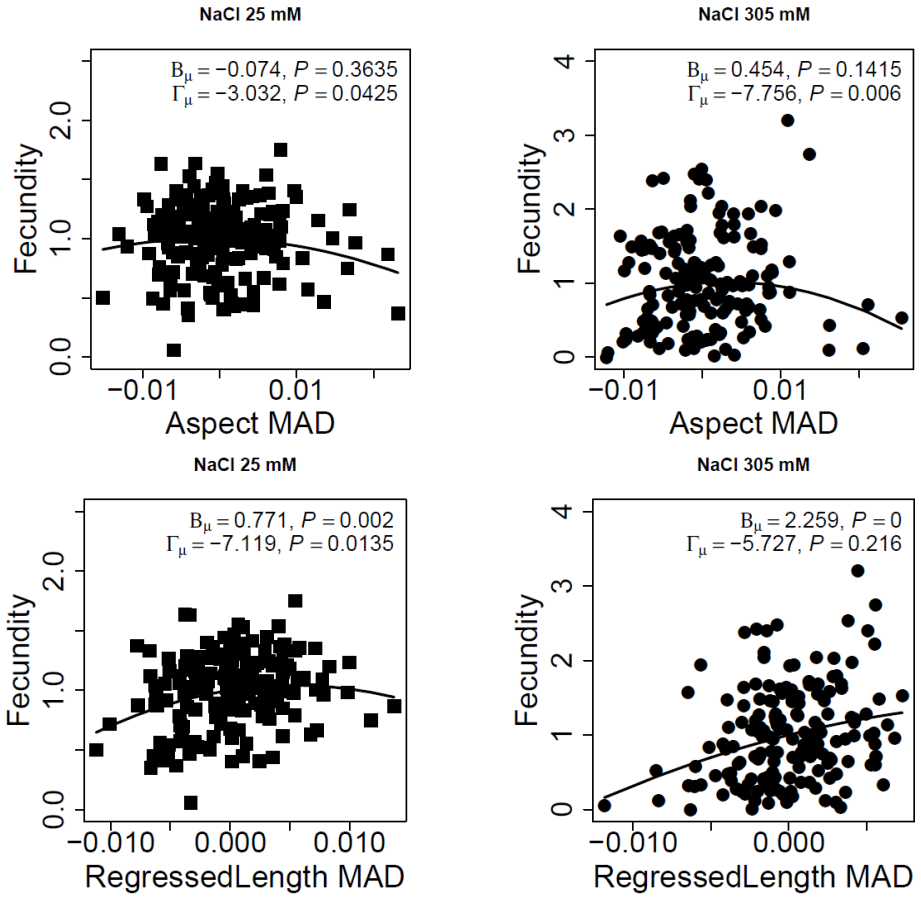
FwdLongitudinalBending	−148.0265244	66.49219892	0.006*
FwdLateralBending	−0.637304481	0.566839381	0.0655
FwdAspect	−114.8494711	47.61530416	0.003*
FwdVelocity	−3.521770072	66.30861423	0.4545
FwdTurningRate	0.005221982	0.006064192	0.1885
FwdCurvature	−0.01404647	0.006989257	0.014*
RevRegLength	−14.33562194	6.792156495	0.0125*
RevRegWidth	−267.4723424	107.3749833	0.0045*
RevLongitudinalBending	−118.4001721	62.64899547	0.016*
RevLateralBending	−0.672730425	0.538325549	0.0485*
RevAspect	−106.2750873	50.55026129	0.0105*
RevVelocity	−33.35333075	87.32479095	0.328
RevTurningRate	0.00610832	0.003587194	0.043*
RevCurvature	−0.01099341	0.007168797	0.0315*
StatRegLength	−15.01679458	6.005888293	0.004*
StatRegWidth	−159.9109477	87.72535468	0.0255*
StatLongitudinalBending	−117.7888101	53.46395452	0.0035*
StatLateralBending	−0.555655616	0.562814367	0.1
StatAspect	−91.76905923	38.18581713	0.003*
StatTurningRate	0.01496728	0.179179442	0.4605
StatCurvature	−0.01069656	0.006607321	0.0275*
FwdDuration	−0.26808506	0.184961155	0.05
FwdInterval	−0.010588233	0.022854409	0.3015
RevDuration	−0.214220115	0.608072548	0.336
RevInterval	0.00577665	0.01812398	0.3825
BoutInterval	−0.017039771	0.018195618	0.14
FwdFrequency	−0.01552859	0.009152671	0.023*
FwdFraction	−6.85165270	3.05038944	0.005*
FwdDistance	−0.229338404	0.113405311	0.0115*
RevFrequency	−0.07283098	0.03715649	0.016*
RevFraction	−150.2679963	74.37997171	0.0115*
RevDistance	−5.586548439	2.742090081	0.0125*
LocomotionFraction	−5.29338696	2.411492764	0.0035*
TotalDistanceTravelled	−0.18452599	0.088690912	0.0055*
NetDistanceTravelled	−0.254005487	0.142414642	0.0295*
ExplorationRate	−0.434836283	0.279559292	0.0485*
ExplorationDensity	−0.03356899	0.029098821	0.0805
DistanceNearestAvgNeighbour	−0.886552471	0.353710104	0.0055*
DistanceNearestAvgNeighbourStd	1.98×10^{-07}	8.77×10^{-07}	0.403

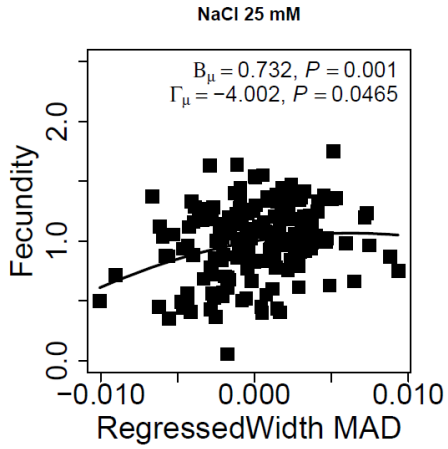
Feature	Γ_{305}	Standard error	*P*
Aspect	−104.0202196	71.90419927	0.0375*
RegressedLength	16.29393867	20.04979062	0.177
RegressedWidth	−462.0751821	279.3574507	0.0165*
Velocity	−402.7822418	675.9735113	0.2795
TurningRate	0.08556157	0.187854193	0.292
Curvature	−0.007971323	0.01969127	0.3045
FwdRegLength	14.16215195	22.55720457	0.2245
FwdRegWidth	−572.927741	299.426604	0.0065*
FwdLongitudinalBending	−219.2504139	102.6416425	0.004*
FwdLateralBending	1.309602294	1.372122647	0.111
FwdAspect	−122.2514955	80.44524656	0.029*
FwdVelocity	28.08708022	69.44873601	0.306
FwdTurningRate	0.006860119	0.004144461	0.035*
FwdCurvature	−0.015736951	0.020633921	0.1535
RevRegLength	5.01887965	23.7769221	0.3955
RevRegWidth	−468.3274539	243.7658704	0.0095*
RevLongitudinalBending	−184.4491756	77.25839775	0.002*
RevLateralBending	1.02610940	0.955941614	0.096
RevAspect	−148.4229345	84.19901867	0.013*
RevVelocity	66.29679221	61.40333604	0.11
RevTurningRate	0.004854043	0.001712122	0.006*
RevCurvature	−0.016840621	0.012129408	0.046*
StatRegLength	17.15608259	20.36079403	0.1695
StatRegWidth	−479.2260246	266.1775287	0.0105*
StatLongitudinalBending	−187.9327132	97.1679373	0.009*
StatLateralBending	2.885644806	1.572188124	0.012*
StatAspect	−98.32572421	68.85634271	0.0365*
StatTurningRate	0.244296568	0.181129261	0.079
StatCurvature	−0.003021419	0.019219363	0.4125
FwdDuration	−0.548551656	0.426800085	0.0635
FwdInterval	−0.062015564	0.079733727	0.1615
RevDuration	−0.667474053	0.75482122	0.14
RevInterval	−0.009145989	0.026210474	0.326
BoutInterval	−0.017420389	0.034539597	0.2995
FwdFrequency	−0.026187044	0.012050893	0.002*
FwdFraction	−12.64136304	7.714187525	0.009*
FwdDistance	−0.86341267	0.41370103	0.004*
RevFrequency	−0.137175841	0.058570849	0.002*
RevFraction	−296.0371377	162.8602377	0.005*
RevDistance	−15.52710138	8.019714002	0.007*
LocomotionFraction	−8.83353955	5.532863932	0.01*
TotalDistanceTravelled	−0.614583868	0.294340095	0.0035*
NetDistanceTravelled	−1.155566675	0.597610291	0.007*
ExplorationRate	0.025358937	1.82059764	0.4735
ExplorationDensity	−0.084637542	0.09471219	0.1215
DistanceNearestAvgNeighbour	0.102000579	0.10556639	0.164

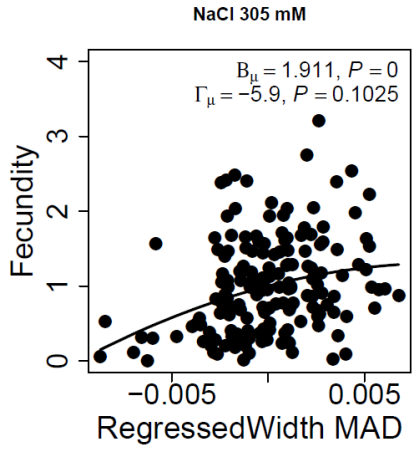
DistanceNearestAvgNeighbourStd	2.83×10^{-06}	2.22×10^{-06}	0.0605

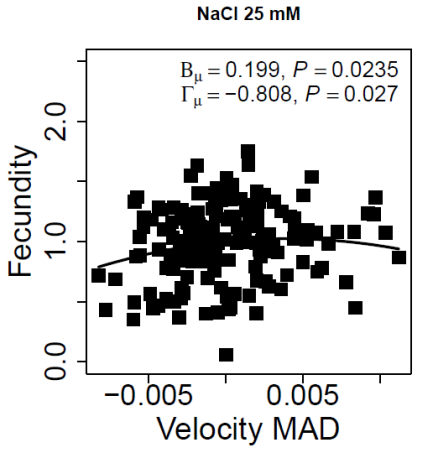
Appendix 3.2: Univariate elasticities of selection on behaviour variability features.

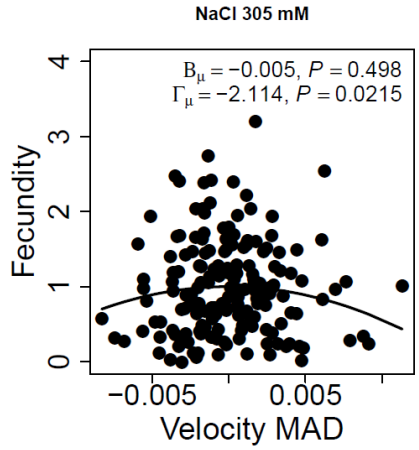
Only state-based and event-based behavioural features are under analysis here, as cumulative-based features will have no variability by definition (see Chapter 2).

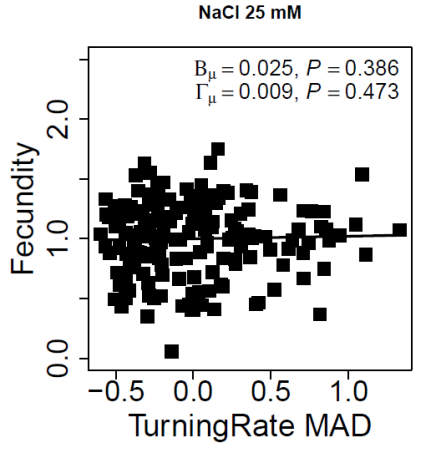


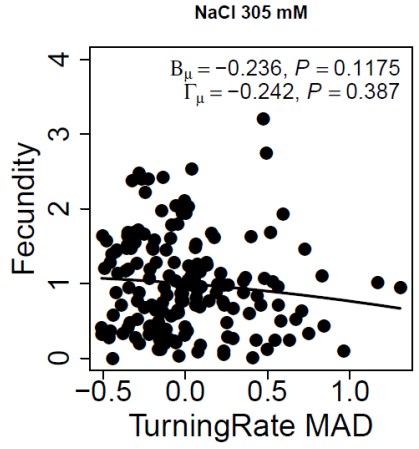

NaCl 25 mM
$B_\mu = 0.732$, $P = 0.001$
$\Gamma_\mu = -4.002$, $P = 0.0465$
Fecundity
RegressedWidth MAD

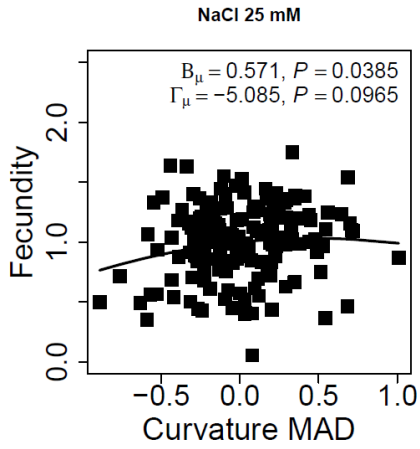

NaCl 305 mM
$B_\mu = 1.911$, $P = 0$
$\Gamma_\mu = -5.9$, $P = 0.1025$
Fecundity
RegressedWidth MAD

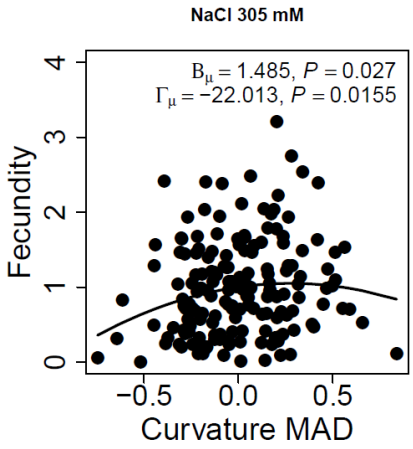

NaCl 25 mM
$B_\mu = 0.199$, $P = 0.0235$
$\Gamma_\mu = -0.808$, $P = 0.027$
Fecundity
Velocity MAD

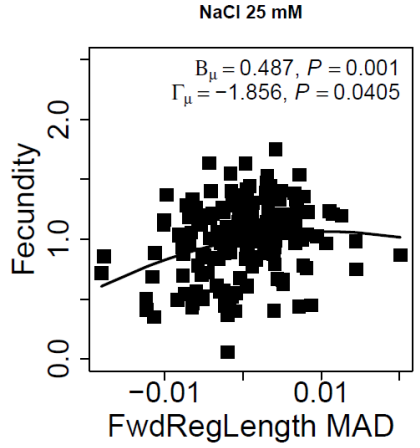

NaCl 305 mM
$B_\mu = -0.005$, $P = 0.498$
$\Gamma_\mu = -2.114$, $P = 0.0215$
Fecundity
Velocity MAD

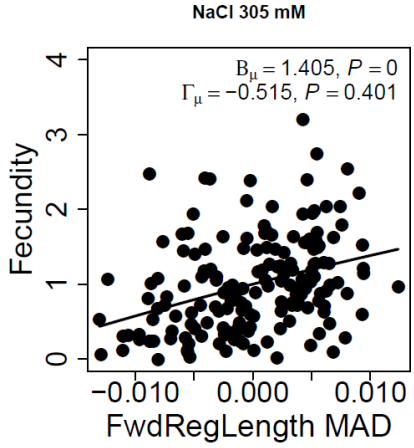

NaCl 25 mM
$B_\mu = 0.025$, $P = 0.386$
$\Gamma_\mu = 0.009$, $P = 0.473$
Fecundity
TurningRate MAD

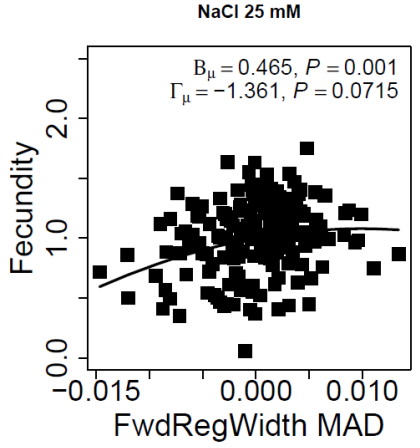

NaCl 305 mM
$B_\mu = -0.236$, $P = 0.1175$
$\Gamma_\mu = -0.242$, $P = 0.387$
Fecundity
TurningRate MAD

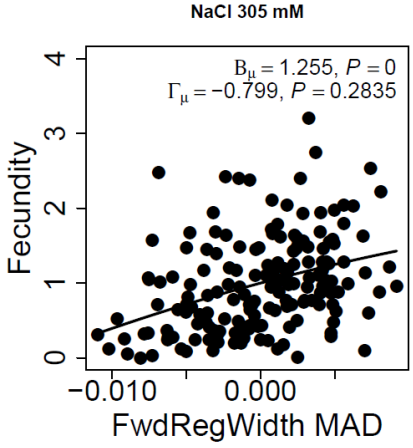

NaCl 25 mM
$B_\mu = 0.571$, $P = 0.0385$
$\Gamma_\mu = -5.085$, $P = 0.0965$
Fecundity
Curvature MAD

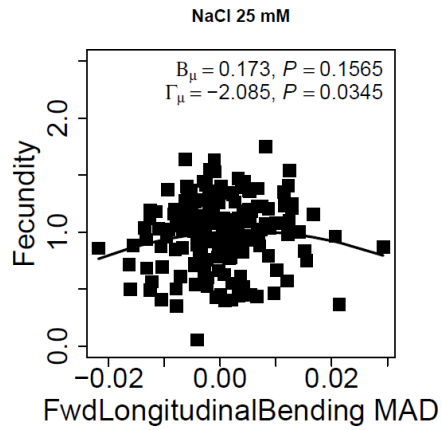

NaCl 305 mM
$B_\mu = 1.485$, $P = 0.027$
$\Gamma_\mu = -22.013$, $P = 0.0155$
Fecundity
Curvature MAD

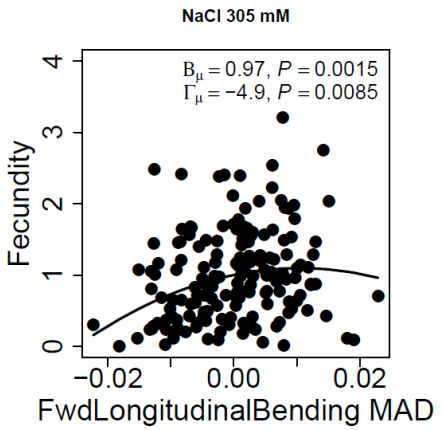

NaCl 25 mM
$B_\mu = 0.487$, $P = 0.001$
$\Gamma_\mu = -1.856$, $P = 0.0405$
Fecundity
FwdRegLength MAD

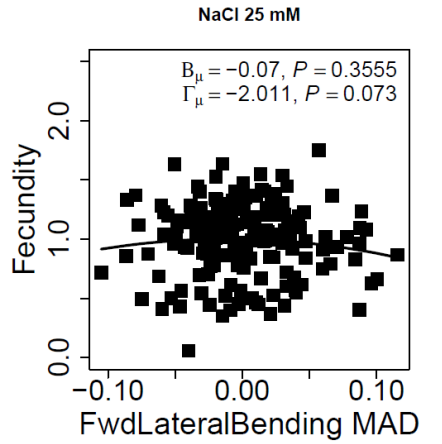

NaCl 305 mM
$B_\mu = 1.405$, $P = 0$
$\Gamma_\mu = -0.515$, $P = 0.401$
Fecundity
FwdRegLength MAD

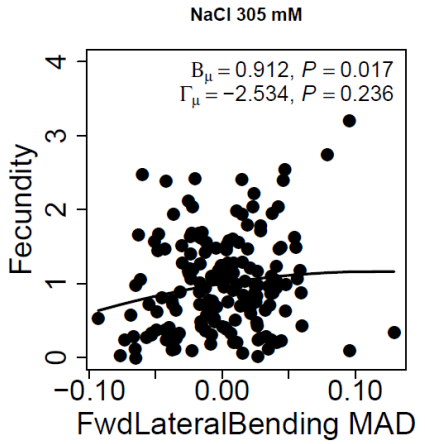

NaCl 25 mM
$B_\mu = 0.465$, $P = 0.001$
$\Gamma_\mu = -1.361$, $P = 0.0715$
Fecundity
FwdRegWidth MAD

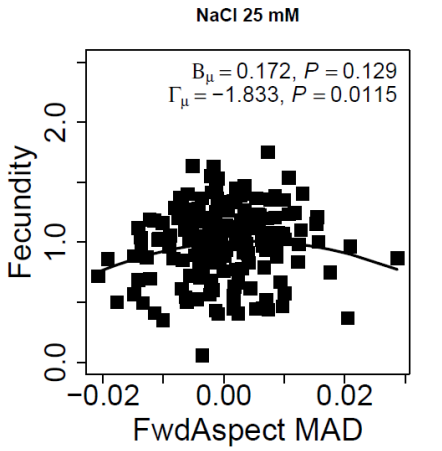

NaCl 305 mM
$B_\mu = 1.255$, $P = 0$
$\Gamma_\mu = -0.799$, $P = 0.2835$
Fecundity
FwdRegWidth MAD

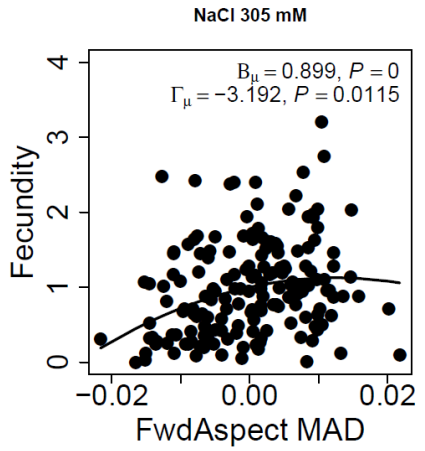

NaCl 25 mM
$B_\mu = 0.173$, $P = 0.1565$
$\Gamma_\mu = -2.085$, $P = 0.0345$
Fecundity
0.0
1.0
2.0
−0.02
0.00
0.02
FwdLongitudinalBending MAD

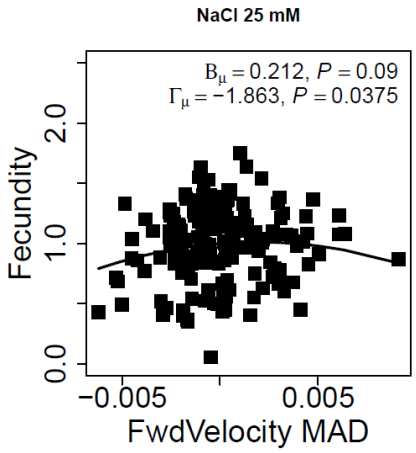

NaCl 305 mM
$B_\mu = 0.97$, $P = 0.0015$
$\Gamma_\mu = -4.9$, $P = 0.0085$
Fecundity
0
1
2
3
4
−0.02
0.00
0.02
FwdLongitudinalBending MAD

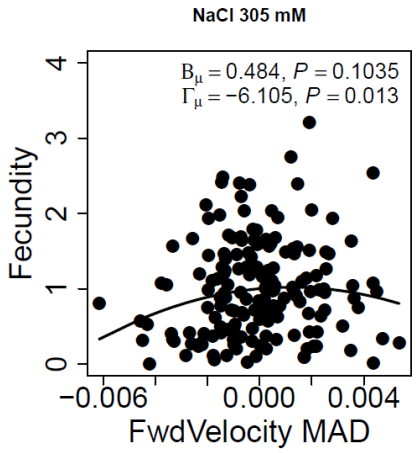

NaCl 25 mM
$B_\mu = -0.07$, $P = 0.3555$
$\Gamma_\mu = -2.011$, $P = 0.073$
Fecundity
0.0
1.0
2.0
−0.10
0.00
0.10
FwdLateralBending MAD

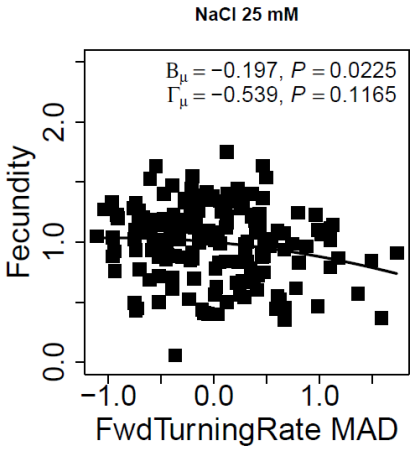

NaCl 305 mM
$B_\mu = 0.912$, $P = 0.017$
$\Gamma_\mu = -2.534$, $P = 0.236$
Fecundity
0
1
2
3
4
−0.10
0.00
0.10
FwdLateralBending MAD

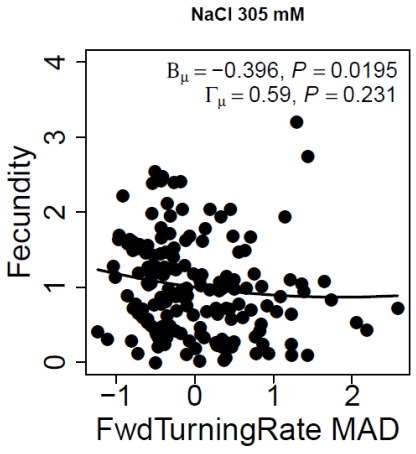

NaCl 25 mM
$B_\mu = 0.172$, $P = 0.129$
$\Gamma_\mu = -1.833$, $P = 0.0115$
Fecundity
0.0
1.0
2.0
−0.02
0.00
0.02
FwdAspect MAD

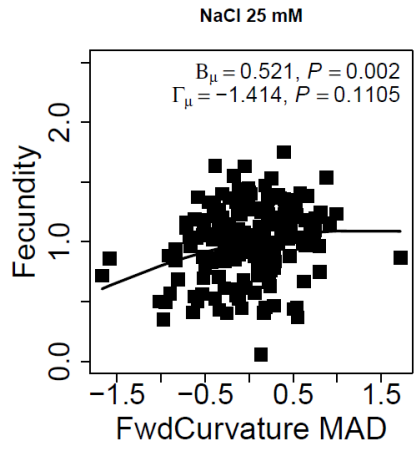

NaCl 305 mM
$B_\mu = 0.899$, $P = 0$
$\Gamma_\mu = -3.192$, $P = 0.0115$
Fecundity
0
1
2
3
4
−0.02
0.00
0.02
FwdAspect MAD

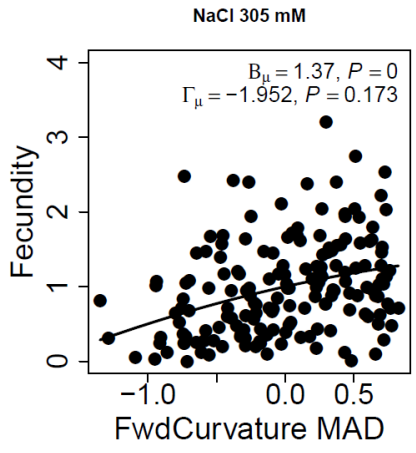

NaCl 25 mM
$B_\mu = 0.212$, $P = 0.09$
$\Gamma_\mu = -1.863$, $P = 0.0375$
Fecundity
FwdVelocity MAD

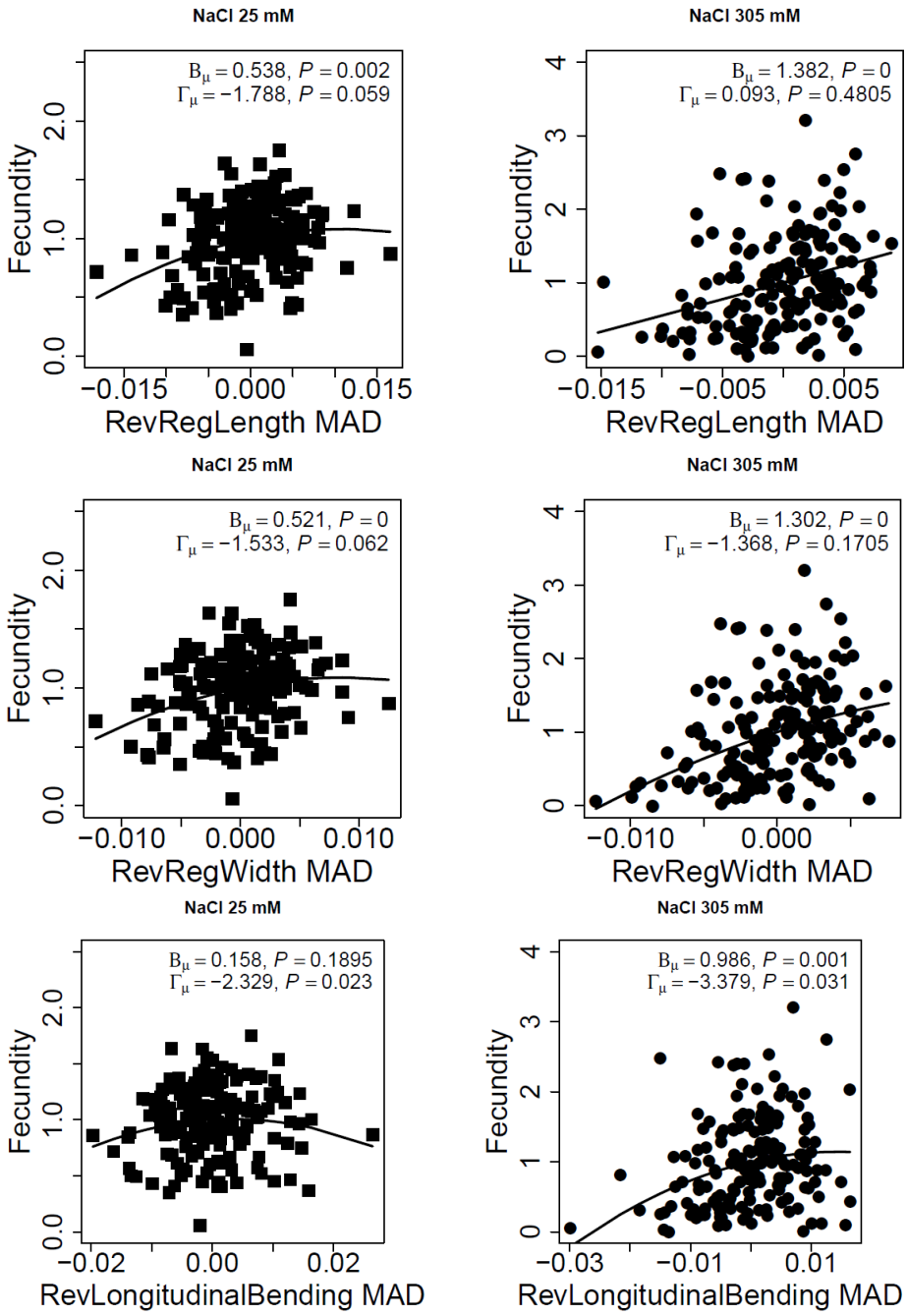

NaCl 305 mM
$B_\mu = 0.484$, $P = 0.1035$
$\Gamma_\mu = -6.105$, $P = 0.013$
Fecundity
FwdVelocity MAD

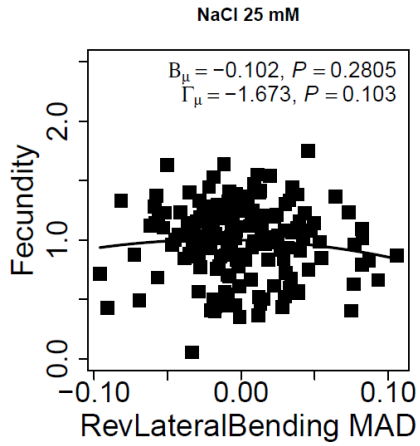

NaCl 25 mM
$B_\mu = -0.197$, $P = 0.0225$
$\Gamma_\mu = -0.539$, $P = 0.1165$
Fecundity
FwdTurningRate MAD

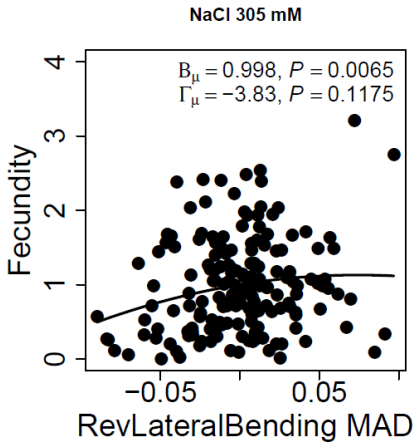

NaCl 305 mM
$B_\mu = -0.396$, $P = 0.0195$
$\Gamma_\mu = 0.59$, $P = 0.231$
Fecundity
FwdTurningRate MAD

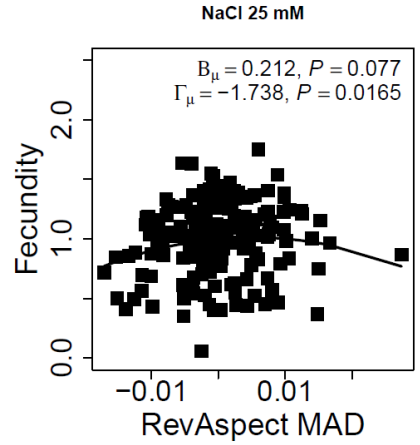

NaCl 25 mM
$B_\mu = 0.521$, $P = 0.002$
$\Gamma_\mu = -1.414$, $P = 0.1105$
Fecundity
FwdCurvature MAD

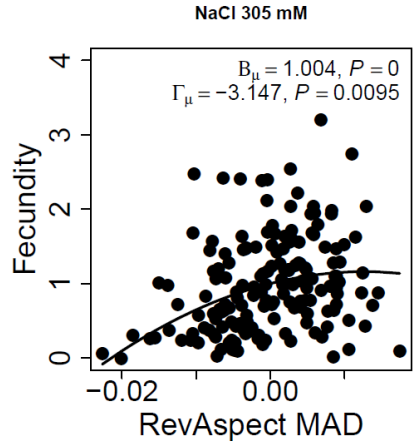

NaCl 305 mM
$B_\mu = 1.37$, $P = 0$
$\Gamma_\mu = -1.952$, $P = 0.173$
Fecundity
FwdCurvature MAD

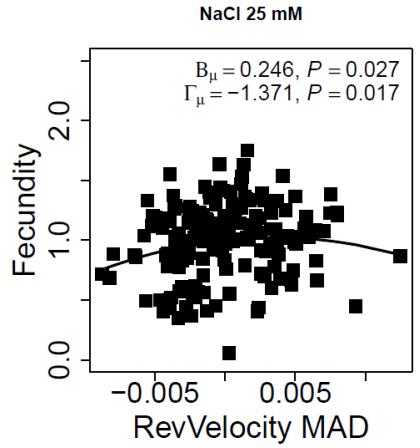

NaCl 25 mM
$B_\mu = 0.538$, $P = 0.002$
$\Gamma_\mu = -1.788$, $P = 0.059$
Fecundity
RevRegLength MAD
NaCl 305 mM
$B_\mu = 1.382$, $P = 0$
$\Gamma_\mu = 0.093$, $P = 0.4805$
Fecundity
RevRegLength MAD
NaCl 25 mM
$B_\mu = 0.521$, $P = 0$
$\Gamma_\mu = -1.533$, $P = 0.062$
Fecundity
RevRegWidth MAD
NaCl 305 mM
$B_\mu = 1.302$, $P = 0$
$\Gamma_\mu = -1.368$, $P = 0.1705$
Fecundity
RevRegWidth MAD
NaCl 25 mM
$B_\mu = 0.158$, $P = 0.1895$
$\Gamma_\mu = -2.329$, $P = 0.023$
Fecundity
RevLongitudinalBending MAD
NaCl 305 mM
$B_\mu = 0.986$, $P = 0.001$
$\Gamma_\mu = -3.379$, $P = 0.031$
Fecundity
RevLongitudinalBending MAD

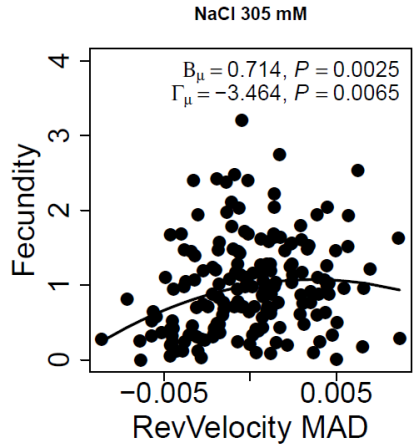

NaCl 25 mM
$B_\mu = -0.102$, $P = 0.2805$
$\Gamma_\mu = -1.673$, $P = 0.103$
Fecundity
0.0
1.0
2.0
−0.10
0.00
0.10
RevLateralBending MAD

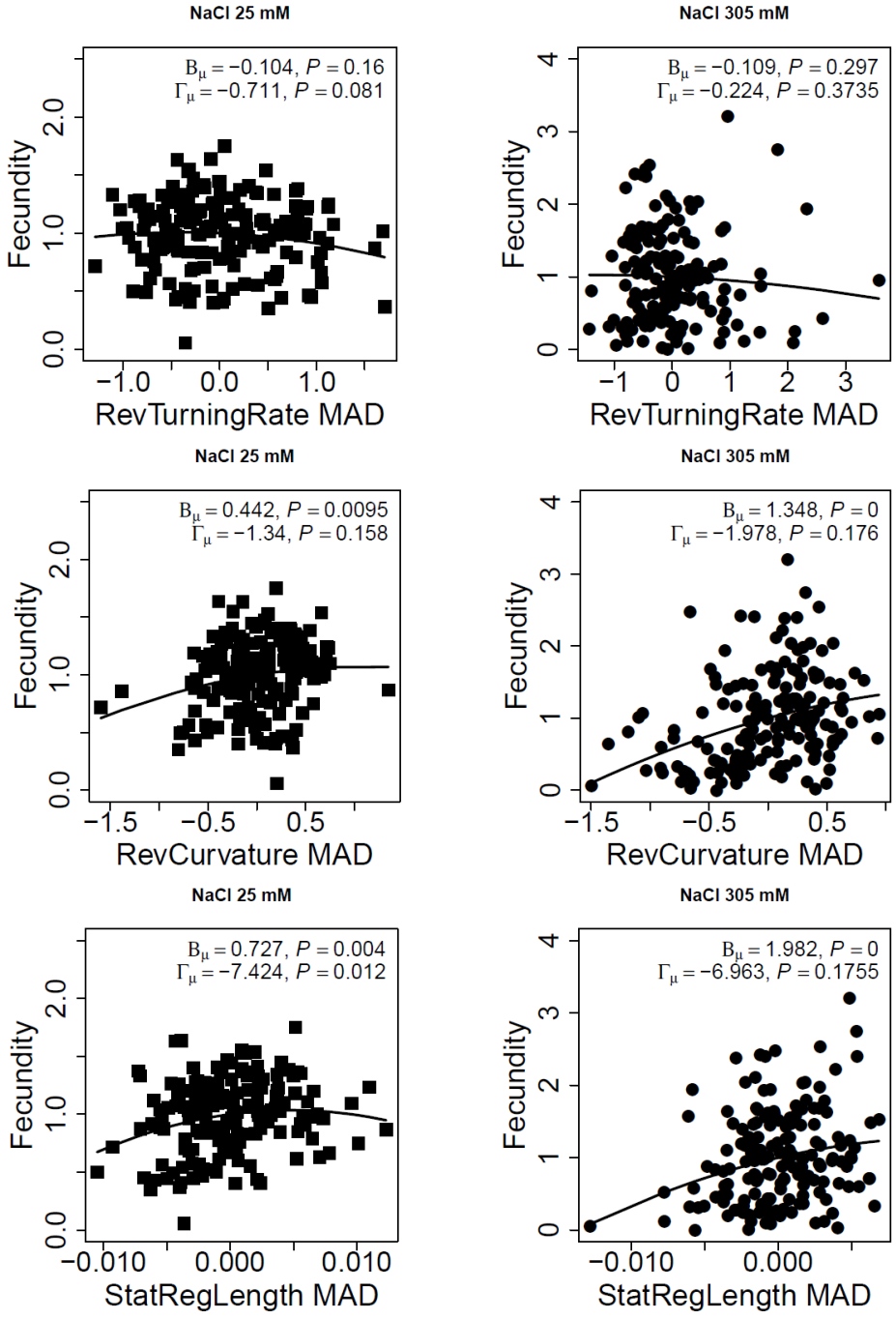

NaCl 305 mM
$B_\mu = 0.998$, $P = 0.0065$
$\Gamma_\mu = -3.83$, $P = 0.1175$
Fecundity
0
1
2
3
4
−0.05
0.05
RevLateralBending MAD

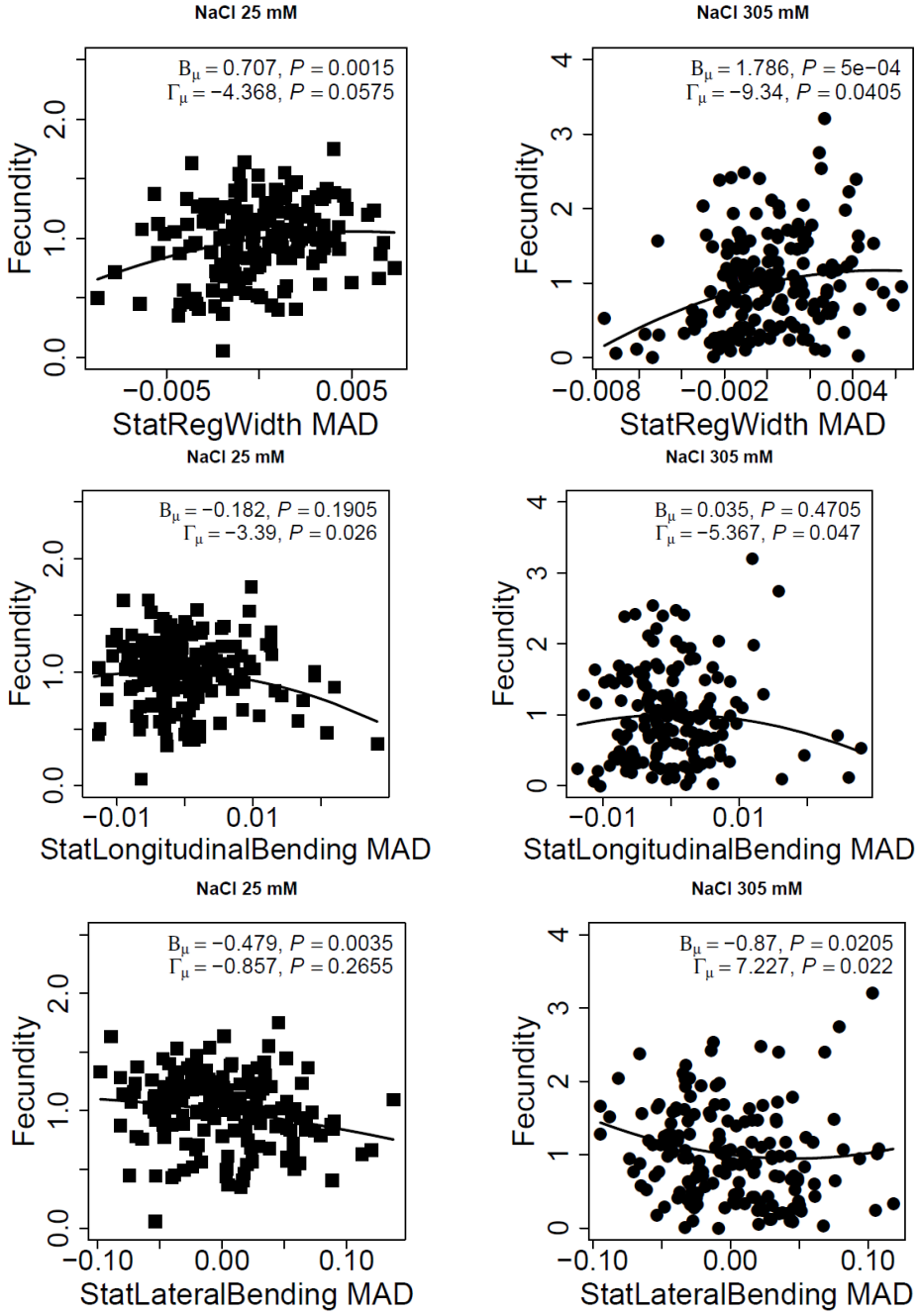

NaCl 25 mM
$B_\mu = 0.212$, $P = 0.077$
$\Gamma_\mu = -1.738$, $P = 0.0165$
Fecundity
0.0
1.0
2.0
−0.01
0.01
RevAspect MAD

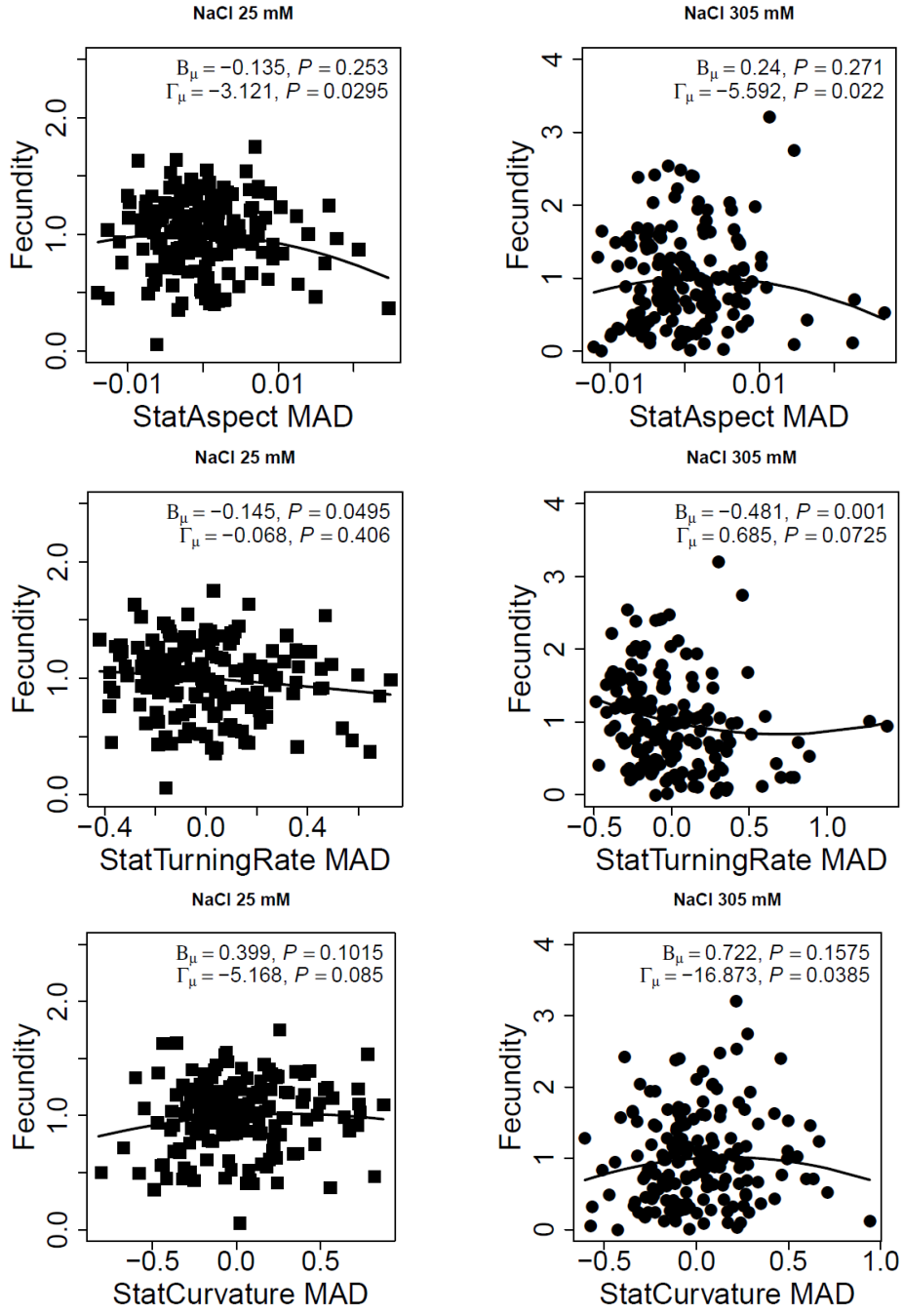

NaCl 305 mM
$B_\mu = 1.004$, $P = 0$
$\Gamma_\mu = -3.147$, $P = 0.0095$
Fecundity
0
1
2
3
4
−0.02
0.00
RevAspect MAD

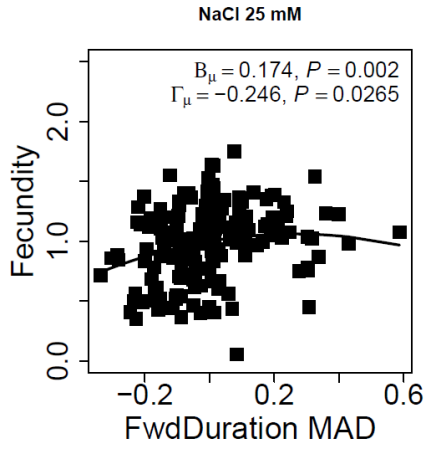

NaCl 25 mM
$B_\mu = 0.246$, $P = 0.027$
$\Gamma_\mu = -1.371$, $P = 0.017$
Fecundity
0.0
1.0
2.0
−0.005
0.005
RevVelocity MAD

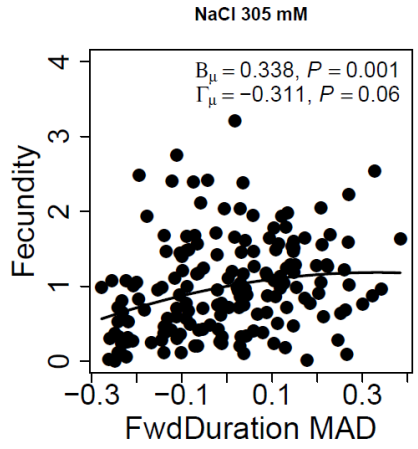

NaCl 305 mM
$B_\mu = 0.714$, $P = 0.0025$
$\Gamma_\mu = -3.464$, $P = 0.0065$
Fecundity
0
1
2
3
4
−0.005
0.005
RevVelocity MAD

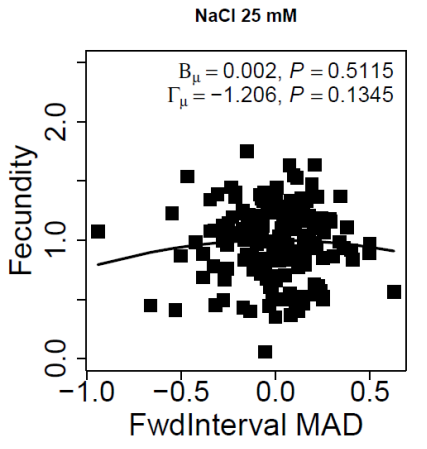

NaCl 25 mM
$B_\mu = -0.104$, $P = 0.16$
$\Gamma_\mu = -0.711$, $P = 0.081$
Fecundity
RevTurningRate MAD
NaCl 305 mM
$B_\mu = -0.109$, $P = 0.297$
$\Gamma_\mu = -0.224$, $P = 0.3735$
Fecundity
RevTurningRate MAD
NaCl 25 mM
$B_\mu = 0.442$, $P = 0.0095$
$\Gamma_\mu = -1.34$, $P = 0.158$
Fecundity
RevCurvature MAD
NaCl 305 mM
$B_\mu = 1.348$, $P = 0$
$\Gamma_\mu = -1.978$, $P = 0.176$
Fecundity
RevCurvature MAD
NaCl 25 mM
$B_\mu = 0.727$, $P = 0.004$
$\Gamma_\mu = -7.424$, $P = 0.012$
Fecundity
StatRegLength MAD
NaCl 305 mM
$B_\mu = 1.982$, $P = 0$
$\Gamma_\mu = -6.963$, $P = 0.1755$
Fecundity
StatRegLength MAD

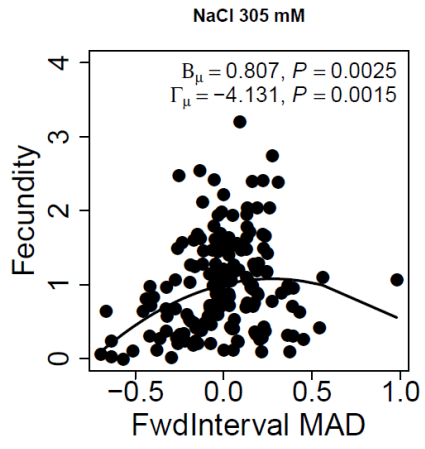

NaCl 25 mM
$B_\mu = 0.707$, $P = 0.0015$
$\Gamma_\mu = -4.368$, $P = 0.0575$
Fecundity
StatRegWidth MAD
NaCl 305 mM
$B_\mu = 1.786$, $P = 5e{-}04$
$\Gamma_\mu = -9.34$, $P = 0.0405$
Fecundity
StatRegWidth MAD
NaCl 25 mM
$B_\mu = -0.182$, $P = 0.1905$
$\Gamma_\mu = -3.39$, $P = 0.026$
Fecundity
StatLongitudinalBending MAD
NaCl 305 mM
$B_\mu = 0.035$, $P = 0.4705$
$\Gamma_\mu = -5.367$, $P = 0.047$
Fecundity
StatLongitudinalBending MAD
NaCl 25 mM
$B_\mu = -0.479$, $P = 0.0035$
$\Gamma_\mu = -0.857$, $P = 0.2655$
Fecundity
StatLateralBending MAD
NaCl 305 mM
$B_\mu = -0.87$, $P = 0.0205$
$\Gamma_\mu = 7.227$, $P = 0.022$
Fecundity
StatLateralBending MAD

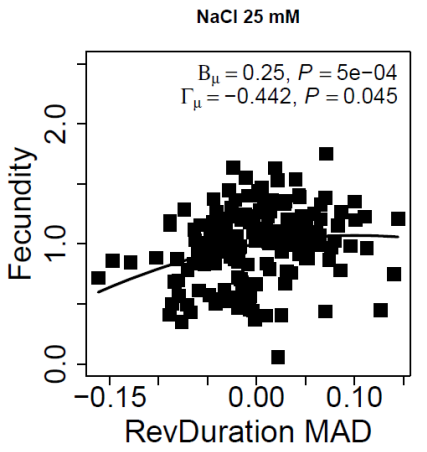

NaCl 25 mM
$B_\mu = -0.135$, $P = 0.253$
$\Gamma_\mu = -3.121$, $P = 0.0295$
Fecundity
StatAspect MAD
NaCl 305 mM
$B_\mu = 0.24$, $P = 0.271$
$\Gamma_\mu = -5.592$, $P = 0.022$
Fecundity
StatAspect MAD
NaCl 25 mM
$B_\mu = -0.145$, $P = 0.0495$
$\Gamma_\mu = -0.068$, $P = 0.406$
Fecundity
StatTurningRate MAD
NaCl 305 mM
$B_\mu = -0.481$, $P = 0.001$
$\Gamma_\mu = 0.685$, $P = 0.0725$
Fecundity
StatTurningRate MAD
NaCl 25 mM
$B_\mu = 0.399$, $P = 0.1015$
$\Gamma_\mu = -5.168$, $P = 0.085$
Fecundity
StatCurvature MAD
NaCl 305 mM
$B_\mu = 0.722$, $P = 0.1575$
$\Gamma_\mu = -16.873$, $P = 0.0385$
Fecundity
StatCurvature MAD

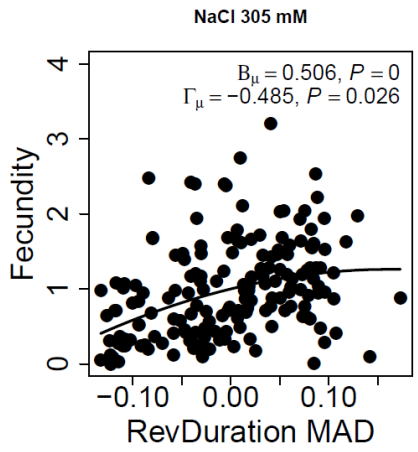

NaCl 25 mM
$B_\mu = 0.174$, $P = 0.002$
$\Gamma_\mu = -0.246$, $P = 0.0265$
Fecundity
FwdDuration MAD

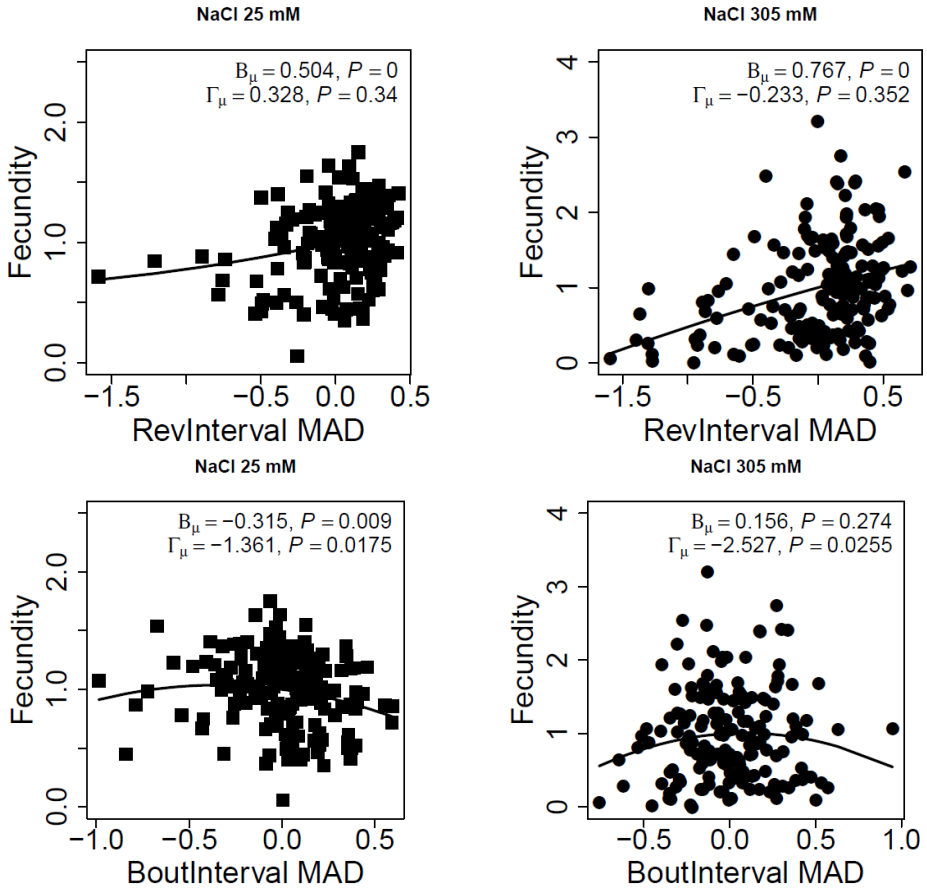

NaCl 305 mM
$B_\mu = 0.338$, $P = 0.001$
$\Gamma_\mu = -0.311$, $P = 0.06$
Fecundity
FwdDuration MAD


NaCl 25 mM
$B_\mu = 0.002$, $P = 0.5115$
$\Gamma_\mu = -1.206$, $P = 0.1345$
Fecundity
FwdInterval MAD


NaCl 305 mM
$B_\mu = 0.807$, $P = 0.0025$
$\Gamma_\mu = -4.131$, $P = 0.0015$
Fecundity
FwdInterval MAD


NaCl 25 mM
$B_\mu = 0.25$, $P = 5e{-}04$
$\Gamma_\mu = -0.442$, $P = 0.045$
Fecundity
RevDuration MAD


NaCl 305 mM
$B_\mu = 0.506$, $P = 0$
$\Gamma_\mu = -0.485$, $P = 0.026$
Fecundity
RevDuration MAD



Appendix 3.1.2: Univariate selection estimates for behaviour variability.

Below are the estimates of selection gradients (B) and selection curvature (Γ) on behaviour variability (MAD). These estimates also correspond to the elasticities shown above. Subscripts specify the environment, defined by NaCl concentration. Asterisks highlight the statistical significant estimates under a significance level of 0.05 and the *P* values are the same ones that are on the respective plots above.

	B_{25}	Standard error	*P*
AspectMAD	−1.311530041	3.709621629	0.3635
RegressedLengthMAD	15.45071776	5.407130636	0.002*
RegressedWidthMAD	21.41999496	6.902270813	0.001*
VelocityMAD	12.49171707	6.22324766	0.0235*
TurningRateMAD	0.018614793	0.057576552	0.386
CurvatureMAD	0.13556016	0.073548814	0.0385*
FwdRegLengthMAD	11.81978431	3.780720603	0.001*
FwdRegWidthMAD	15.9512869	4.976909582	0.001*
FwdLongitudinalBendingMAD	3.150805098	2.997068853	0.1565
FwdLateralBendingMAD	−0.218252928	0.575555432	0.3555
FwdAspectMAD	3.601225503	2.937195373	0.129
FwdVelocityMAD	12.71353707	9.614918812	0.09
FwdTurningRateMAD	−0.078000304	0.039856208	0.0225*
FwdCurvatureMAD	0.14475550	0.045896038	0.002*
RevRegLengthMAD	14.98624791	4.574406086	0.002*
RevRegWidthMAD	20.6567567	5.930640842	0*
RevLongitudinalBendingMAD	3.139040175	3.349201811	0.1895
RevLateralBendingMAD	−0.362752515	0.640944297	0.2805
RevAspectMAD	5.047141954	3.345364588	0.077
RevVelocityMAD	12.52177406	6.216066004	0.027*
RevTurningRateMAD	−0.038396056	0.038384819	0.16
RevCurvatureMAD	0.1350302	0.056444652	0.0095*
StatRegLengthMAD	14.86701717	5.763957442	0.004*
StatRegWidthMAD	21.56812946	7.855717617	0.0015*
StatLongitudinalBendingMAD	−2.939057796	3.282785093	0.1905
StatLateralBendingMAD	−1.32675643	0.508820646	0.0035*
StatAspectMAD	−2.449539495	3.687793241	0.253
StatTurningRateMAD	−0.16194613	0.09865468	0.0495*
StatCurvatureMAD	0.096840169	0.074568919	0.1015
FwdDurationMAD	0.465778578	0.144802812	0.002*
FwdIntervalMAD	0.001180417	0.104387548	0.5115
RevDurationMAD	1.38643971	0.423629852	0.0005*
RevIntervalMAD	0.26925376	0.076962123	0*
BoutIntervalMAD	−0.21050400	0.086270996	0.009*

	B_{305}	Standard error	*P*
AspectMAD	8.245038563	7.698017408	0.1415
RegressedLengthMAD	52.5606759	14.3305186	0*
RegressedWidthMAD	66.5728044	16.93520495	0*
VelocityMAD	−0.318414974	15.19173559	0.498
TurningRateMAD	−0.171257405	0.140454026	0.1175
CurvatureMAD	0.365458872	0.182395732	0.027*
FwdRegLengthMAD	40.45409071	8.457195036	0*
FwdRegWidthMAD	53.33585515	10.52249999	0*
FwdLongitudinalBendingMAD	18.3388701	5.903714839	0.0015*

FwdLateralBendingMAD	2.80703571	1.310805512	0.017*
FwdAspectMAD	20.1872617	5.730596932	0*
FwdVelocityMAD	31.17891085	24.54486192	0.1035
FwdTurningRateMAD	−0.14263501	0.06930332	0.0195*
FwdCurvatureMAD	0.41045554	0.090868698	0*
RevRegLengthMAD	45.18197822	10.19373919	0*
RevRegWidthMAD	63.96353107	12.38529814	0*
RevLongitudinalBendingMAD	19.89853202	6.35784455	0.001*
RevLateralBendingMAD	3.498998639	1.431335226	0.0065*
RevAspectMAD	25.27711041	6.401803556	0*
RevVelocityMAD	40.1871627	14.5256627	0.0025*
RevTurningRateMAD	−0.03711469	0.067344959	0.297
RevCurvatureMAD	0.44173651	0.10116197	0*
StatRegLengthMAD	46.82598957	15.19609128	0*
StatRegWidthMAD	64.31260603	19.18170819	0.0005*
StatLongitudinalBendingMAD	0.569838195	7.238920606	0.4705
StatLateralBendingMAD	−2.288903032	1.112368418	0.0205*
StatAspectMAD	4.443033442	7.635562622	0.271
StatTurningRateMAD	−0.47796884	0.152744374	0.001*
StatCurvatureMAD	0.18144617	0.18579147	0.1575
FwdDurationMAD	1.10573509	0.301965954	0.001*
FwdIntervalMAD	0.574788926	0.194962056	0.0025*
RevDurationMAD	3.18675461	0.657525167	0*
RevIntervalMAD	0.478018681	0.101017872	0*
BoutIntervalMAD	0.109324142	0.183372413	0.274

	Γ_{25}	**Standard error**	***P***
AspectMAD	−957.8764725	615.0340217	0.0425*
RegressedLengthMAD	−2858.687169	1448.795241	0.0135*
RegressedWidthMAD	−3425.922065	2311.382399	0.0465*
VelocityMAD	−3196.868266	1929.493278	0.027*
TurningRateMAD	0.005030609	0.181188538	0.473
CurvatureMAD	−0.2867862	0.262326004	0.0965
FwdRegLengthMAD	−1092.648426	664.9645376	0.0405*
FwdRegWidthMAD	−1600.602956	1164.386745	0.0715
FwdLongitudinalBendingMAD	−693.1408751	399.3883802	0.0345*
FwdLateralBendingMAD	−19.39610351	16.31184689	0.073
FwdAspectMAD	−800.6359296	385.8181198	0.0115*
FwdVelocityMAD	−6698.90486	4113.667942	0.0375*
FwdTurningRateMAD	−0.084160654	0.084954852	0.1165
FwdCurvatureMAD	−0.10898476	0.096063898	0.1105
RevRegLengthMAD	−1385.195578	930.9528396	0.059
RevRegWidthMAD	−2410.384311	1642.843679	0.062
RevLongitudinalBendingMAD	−913.6481542	500.762953	0.023*
RevLateralBendingMAD	−21.24677709	19.56769162	0.103
RevAspectMAD	−983.4220482	498.9045215	0.0165*

RevVelocityMAD	−3552.50047	1976.861231	0.017*
RevTurningRateMAD	−0.096949524	0.080705451	0.081
RevCurvatureMAD	−0.125353638	0.136174585	0.158
StatRegLengthMAD	−3103.326568	1658.015994	0.012*
StatRegWidthMAD	−4064.708537	3085.443807	0.0575
StatLongitudinalBendingMAD	−880.2451408	494.8656111	0.026*
StatLateralBendingMAD	−6.567372252	13.89385547	0.2655
StatAspectMAD	−1031.947991	600.7966868	0.0295*
StatTurningRateMAD	−0.084845121	0.50998473	0.406
StatCurvatureMAD	−0.304419697	0.277224054	0.085
FwdDurationMAD	−1.769003487	1.029314771	0.0265*
FwdIntervalMAD	−0.464594063	0.456520258	0.1345
RevDurationMAD	−13.6091819	8.809804748	0.045*
RevIntervalMAD	0.093296133	0.229366787	0.34
BoutIntervalMAD	−0.605918392	0.331036052	0.0175*

	Γ_{305}	**Standard error**	***P***
AspectMAD	-2561.13845	1234.909902	0.006*
RegressedLengthMAD	-3099.843299	4805.034128	0.216
RegressedWidthMAD	-7157.865539	6579.240411	0.1025
VelocityMAD	-8768.87278	4969.25952	0.0215*
TurningRateMAD	-0.126913395	0.469585664	0.387
CurvatureMAD	-1.333740125	0.779506234	0.0155*
FwdRegLengthMAD	-427.1839074	2090.873667	0.401
FwdRegWidthMAD	-1443.884796	3163.556589	0.2835
FwdLongitudinalBendingMAD	-1751.465254	890.9701296	0.0085*
FwdLateralBendingMAD	-24.01283764	39.87345801	0.236
FwdAspectMAD	-1609.929599	883.7708039	0.0115*
FwdVelocityMAD	-25324.68522	13519.33552	0.013*
FwdTurningRateMAD	0.076441752	0.117746588	0.231
FwdCurvatureMAD	-0.175300163	0.246074214	0.173
RevRegLengthMAD	98.97684111	2678.153311	0.4805
RevRegWidthMAD	-3303.354435	3931.851453	0.1705
RevLongitudinalBendingMAD	-1374.89516	887.850432	0.031*
RevLateralBendingMAD	-47.06542582	46.05659463	0.1175
RevAspectMAD	-1995.03483	1027.46675	0.0095*
RevVelocityMAD	-10982.4761	5352.94258	0.0065*
RevTurningRateMAD	-0.026108656	0.086229677	0.3735
RevCurvatureMAD	-0.21249489	0.25957525	0.176
StatRegLengthMAD	-3884.604734	4969.742109	0.1755
StatRegWidthMAD	-12108.41065	8505.047383	0.0405*
StatLongitudinalBendingMAD	-1398.768484	1014.127342	0.047*
StatLateralBendingMAD	50.04813204	31.40015675	0.022*
StatAspectMAD	-1914.16474	1179.91426	0.022*
StatTurningRateMAD	0.675739547	0.488148898	0.0725
StatCurvatureMAD	-1.06649410	0.76950503	0.0385*

FwdDurationMAD	-3.323906158	2.615744978	0.06
FwdIntervalMAD	-2.0977076	0.74512297	0.0015*
RevDurationMAD	-19.265765	12.953565	0.026*
RevIntervalMAD	-0.090341026	0.268023296	0.352
BoutIntervalMAD	-1.24384874	0.737548974	0.0255*

Appendix 4: Linear approximations of the multivariate selection surfaces

P values were calculated using non-parametric null distributions of selection gradients based on 2,000 replicate permutations of fecundity (the fitness proxy) along behavioural values, as done for univariate regressions. Subscripts on the directional selection coefficients (B) specify the environment, defined by NaCl concentration. Asterisks highlight the statistical significant estimates under a significance level of 0.05.

Appendix 4.1: Multivariate selection gradient estimates on features included in the best model after stepwise regression.

Environment is defined by the NaCl concentration and is stated in the subscript of B.

Feature	$\mathbf{B_{25}}$	**Standard error**	***P***
Aspect	−149.957	42.27106	0.002*
RegressedLength	−110.267	30.17718	0.001*
RegressedWidth	231.834	57.4029	0*
Velocity	56.60909	16.97958	0.0065*
FwdRegLength	15.5481	5.569177	0.018*
FwdVelocity	−19.751	10.38893	0.0825
FwdTurningRate	0.22555	0.080827	0.019*
RevVelocity	16.4398	7.912381	0.0655

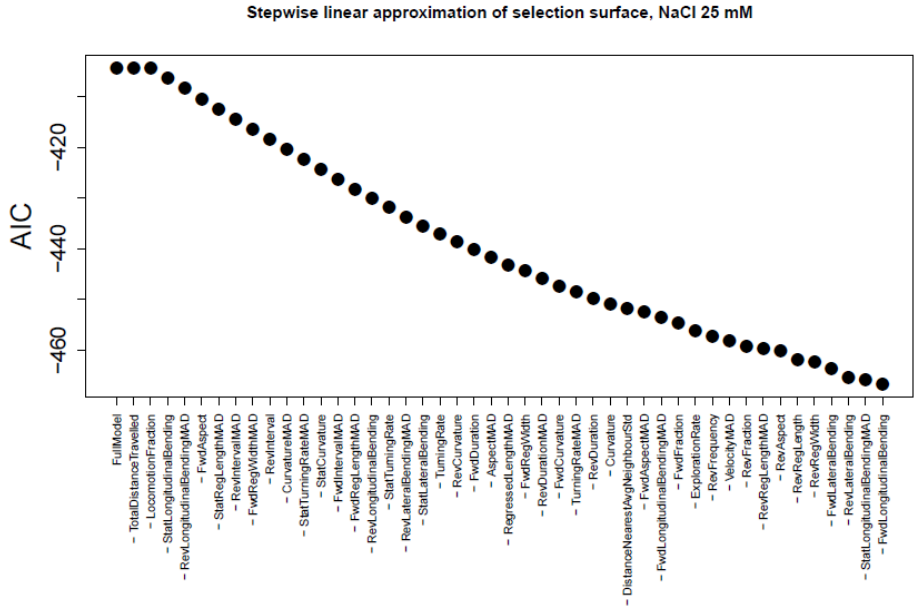
RevTurningRate	−0.14589	0.038767	0.0025*
StatRegLength	86.88555	27.01015	0.0055*
StatRegWidth	−211.744	56.55083	0.0015*
StatAspect	138.3973	41.62382	0.0035*
FwdInterval	0.24216	0.068544	0.0045*
BoutInterval	−0.14295	0.086826	0.1205
FwdFrequency	−0.11145	0.044348	0.029*
FwdDistance	−585.626	361.3845	0.103
RevDistance	584.2139	361.0278	0.1035
NetDistanceTravelled	584.9252	361.2275	0.1035
ExplorationDensity	0.13900	0.059646	0.034*
DistanceNearestAvgNeighbour	0.22028	0.14057	0.118
RegressedWidthMAD	−152.166	58.48811	0.022*
FwdLateralBendingMAD	8.17449	2.566221	0.0055*
FwdVelocityMAD	100.3396	33.39749	0.0105*
FwdTurningRateMAD	−0.85717	0.190855	0.0005*
FwdCurvatureMAD	0.419173	0.231723	0.0975
RevRegWidthMAD	−81.3311	43.7272	0.0765
RevAspectMAD	39.6168	22.2329	0.0945
RevVelocityMAD	−70.0652	27.47704	0.028*
RevTurningRateMAD	0.404726	0.145527	0.019*
RevCurvatureMAD	−0.58202	0.179738	0.0045*
StatRegWidthMAD	298.3973	70.95504	0.001*
StatLateralBendingMAD	−9.58575	2.310524	0.001*
StatAspectMAD	−70.2045	27.21143	0.0275*
StatCurvatureMAD	0.496914	0.211293	0.036*
FwdDurationMAD	−1.2906	0.606247	0.0665
BoutIntervalMAD	−0.8977	0.2058	0*

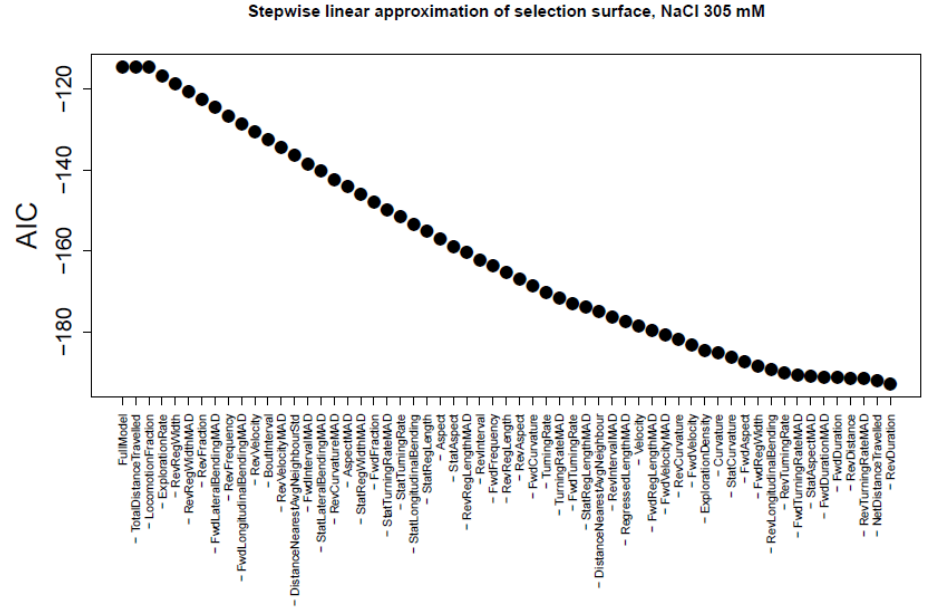
Feature	**B_{305}**	**Standard error**	***P***
RegressedLength	−34.2928	10.35424	0.003*
RegressedWidth	131.7573	77.48979	0.086
FwdRegLength	35.60018	11.39483	0.007*
FwdLongitudinalBending	29.3903	10.17831	0.0095*
FwdLateralBending	−2.48893	1.399157	0.066
RevLateralBending	0.859462	0.582757	0.122
StatRegWidth	−139.468	76.61984	0.068
StatLateralBending	2.648775	1.315988	0.047*
FwdInterval	0.21464	0.089023	0.022*
FwdDistance	−0.85752	0.366012	0.0265*
RegressedWidthMAD	−99.2984	65.55459	0.1185
VelocityMAD	88.16435	35.76503	0.026*
CurvatureMAD	−2.32047	1.070627	0.0345*
FwdRegWidthMAD	140.9358	70.42753	0.054

FwdAspectMAD	−61.0116	34.72209	0.073
FwdCurvatureMAD	1.06014	0.474258	0.0275*
RevLongitudinalBendingMAD	−50.3253	18.00072	0.006*
RevLateralBendingMAD	−6.10323	3.238312	0.065
RevAspectMAD	59.31531	22.73323	0.0175*
StatLongitudinalBendingMAD	−69.0604	29.24377	0.02*
StatCurvatureMAD	2.21470	0.955894	0.0255*
RevDurationMAD	4.003564	2.174565	0.0675
BoutIntervalMAD	−0.41181	0.289594	0.106

Appendix 4.2: Steps involved in the stepwise regressions that have led to the best multivariate linear approximation of the selection surfaces using the original features

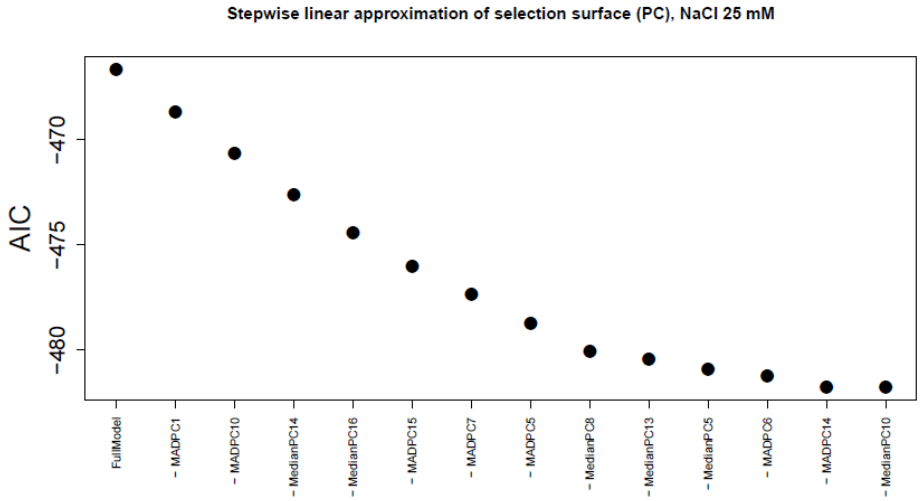
The full model is the model with all linear terms of all features and each step is described in relation to the previous one. For instance, "–RevInterval" refers to a model in which reverse interval was removed relative to a model in which all the terms on the left had been removed from the full model. The Akaike Information Criterion (AIC) is shown for each of the regressions performed.

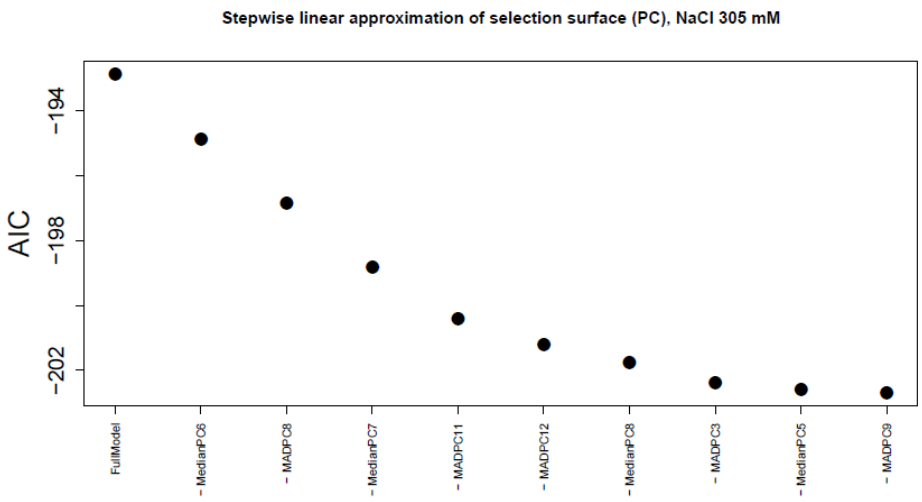

Stepwise linear approximation of selection surface, NaCl 25 mM
AIC
−460
−440
−420
FullModel
− TotalDistanceTravelled
− LocomotionFraction
− StatLongitudinalBending
− RevLongitudinalBendingMAD
− FwdAspect
− StatRegLengthMAD
− RevIntervalMAD
− FwdRegWidthMAD
− RevInterval
− CurvatureMAD
− StatTurningRateMAD
− StatCurvature
− FwdIntervalMAD
− FwdRegLengthMAD
− RevLongitudinalBending
− StatTurningRate
− RevLateralBendingMAD
− StatLateralBending
− TurningRate
− RevCurvature
− FwdDuration
− AspectMAD
− RegressedLengthMAD
− FwdRegWidth
− RevDurationMAD
− FwdCurvature
− TurningRateMAD
− RevDuration
− Curvature
− DistanceNearestAvgNeighbourStd
− FwdAspectMAD
− FwdLongitudinalBendingMAD
− FwdFraction
− ExplorationRate
− RevFrequency
− VelocityMAD
− RevFraction
− RevRegLengthMAD
− RevAspect
− RevRegLength
− RevRegWidth
− FwdLateralBending
− RevLateralBending
− StatLongitudinalBendingMAD
− FwdLongitudinalBending


Stepwise linear approximation of selection surface, NaCl 305 mM
AIC
−180
−160
−140
−120
FullModel
− TotalDistanceTravelled
− LocomotionFraction
− ExplorationRate
− RevRegWidth
− RevRegWidthMAD
− RevFraction
− FwdLateralBendingMAD
− RevFrequency
− FwdLongitudinalBendingMAD
− RevVelocity
− RevInterval
− RevVelocityMAD
− DistanceNearestAvgNeighbourStd
− FwdIntervalMAD
− StatLateralBendingMAD
− RevCurvatureMAD
− AspectMAD
− StatRegWidthMAD
− FwdFraction
− StatTurningRateMAD
− StatTurningRate
− StatLongitudinalBending
− StatRegLength
− Aspect
− StatAspect
− RevRegLengthMAD
− RevInterval
− FwdFrequency
− RevRegLength
− RevAspect
− FwdCurvature
− TurningRate
− TurningRateMAD
− FwdTurningRate
− StatRegLengthMAD
− DistanceNearestAvgNeighbour
− RevIntervalMAD
− RegressedLengthMAD
− Velocity
− FwdRegLengthMAD
− FwdVelocityMAD
− RevCurvature
− FwdVelocity
− ExplorationDensity
− Curvature
− StatCurvature
− FwdRegWidth
− FwdAspect
− RevLongitudinalBending
− RevTurningRate
− FwdTurningRateMAD
− StatAspectMAD
− FwdDurationMAD
− FwdDuration
− RevDistance
− RevTurningRateMAD
− NetDistanceTravelled
− RevDuration

Appendix 4.3: Stepwise regression steps that have led to the best multivariate linear approximation of the selection surface using the principal components of the original features

The original features from which the principal components were obtained were the features retained in the best linear approximation obtained for the respective environment. The full model included all median and MAD principal components and each step was again described in relation to the previous ones in the stepwise regression, as in the previous figures.





Appendix 4.4: Selection gradients of the principal components involved in the linear approximation of the multivariate selection surfaces

In the tables below lie the selection gradient estimates for each principal component both in the full model – the model using all the principal components in each respective environment – and the best model – the best model using principal components after stepwise regression from the full model. The features that contain two estimates are therefore the features maintained in the best model after the stepwise regression performed with the full model as a starting model, being the top estimate the one obtained in the full model and the bottom estimate obtained from the best model.

Feature (PC)	B_{25}	Standard error	*P*
MedianPC1	-0.093788561	0.046137333	0.054
	-0.10384550	0.023284448	0.0005*
MedianPC2	-0.10415906	0.052527538	0.081
	-0.11094153	0.023456188	0.0005*
MedianPC3	0.047171324	0.044733341	0.217

	0.066563946	0.023413905	0.0155*
MedianPC4	0.033581234	0.034937337	0.231
	0.049213796	0.019679867	0.0330*
MedianPC5	-0.046774842	0.034607398	0.1405
MedianPC6	0.085497065	0.046944891	0.0875
	0.059223413	0.032278236	0.0895
MedianPC7	-0.28908258	0.062046294	0*
	-0.23341835	0.03594279	0*
MedianPC8	0.055876414	0.061088199	0.2535
MedianPC9	0.133979214	0.099495869	0.1525
	0.12813338	0.066052175	0.0760
MedianPC10	0.15058773	0.078810389	0.082
MedianPC11	-0.185889182	0.113833038	0.1095
	-0.15466107	0.096406043	0.1155
MedianPC12	-0.257879014	0.110981796	0.0455*
	-0.28599549	0.096513742	0.0150*
MedianPC13	0.197179945	0.176945151	0.2025
MedianPC14	0.043041313	0.212369776	0.4415
MedianPC15	-0.566543101	0.220598186	0.0315*
	-0.367720962	0.184613066	0.0710
MedianPC16	-0.132221309	0.331656546	0.3915
MedianPC17	0.882123029	0.417318686	0.048*
	0.659578252	0.359018093	0.0775
MedianPC18	-2.353683179	1.213924614	0.0615
	-2.107002158	1.060228737	0.0585
MedianPC19	10.5010269	2.866017486	0.001*
	8.575312414	2.576924161	0.0020*
MedianPC20	352.6016356	217.6554251	0.103
	313.3100151	205.1120093	0.1190
MADPC1	0.003641982	0.033243751	0.449
MADPC2	0.22037679	0.066741375	0.006*
	0.25305399	0.040257354	0*
MADPC3	-0.15188232	0.07240979	0.068
	-0.14026228	0.038692816	0.0025*
MADPC4	0.11894953	0.082697578	0.132
	0.14338538	0.047278994	0.0110
MADPC5	-0.027980918	0.043760811	0.317
MADPC6	0.045886365	0.070309747	0.3075
MADPC7	0.060113682	0.080343879	0.286
MADPC8	-0.13430527	0.084084622	0.125
	-0.12137458	0.066696197	0.0875
MADPC9	-0.133861457	0.115118331	0.196
	-0.13716640	0.083173339	0.1100
MADPC10	-0.016144418	0.099467524	0.445
MADPC11	-0.381230678	0.104464548	0.002*
	-0.26158442	0.076913009	0.0070*
MADPC12	-0.28844292	0.093020226	0.009*

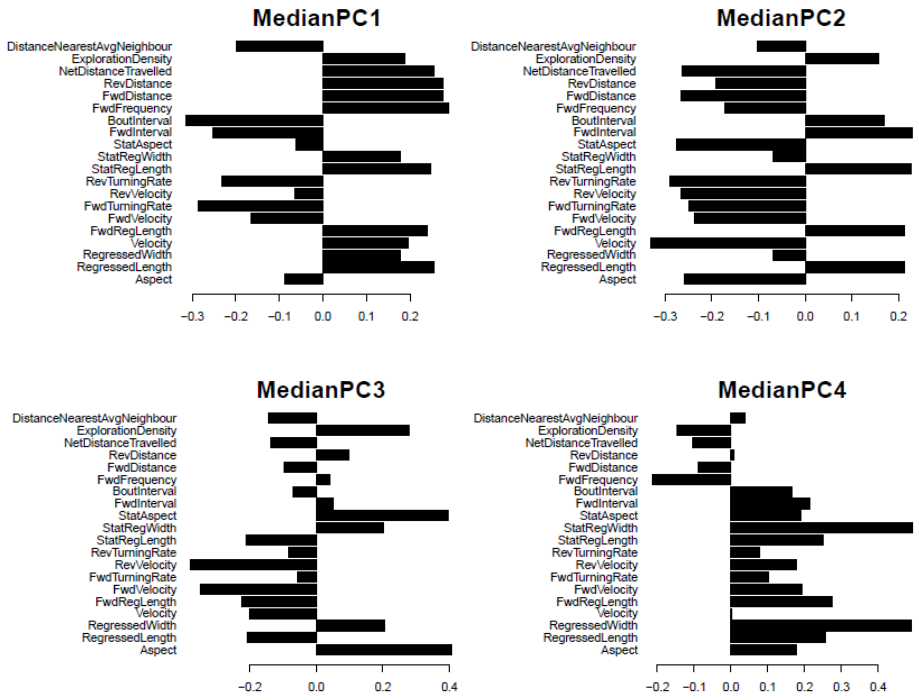
	-0.28949116	0.082143155	0.0025*
MADPC13	0.879988873	0.148526173	0*
	0.680894233	0.112839707	0*
MADPC14	0.242035043	0.16380971	0.1275
MADPC15	-0.155673977	0.228763198	0.2945
MADPC16	-1.06292898	0.273192609	0.0005*
	-0.972196357	0.244482269	0.002*

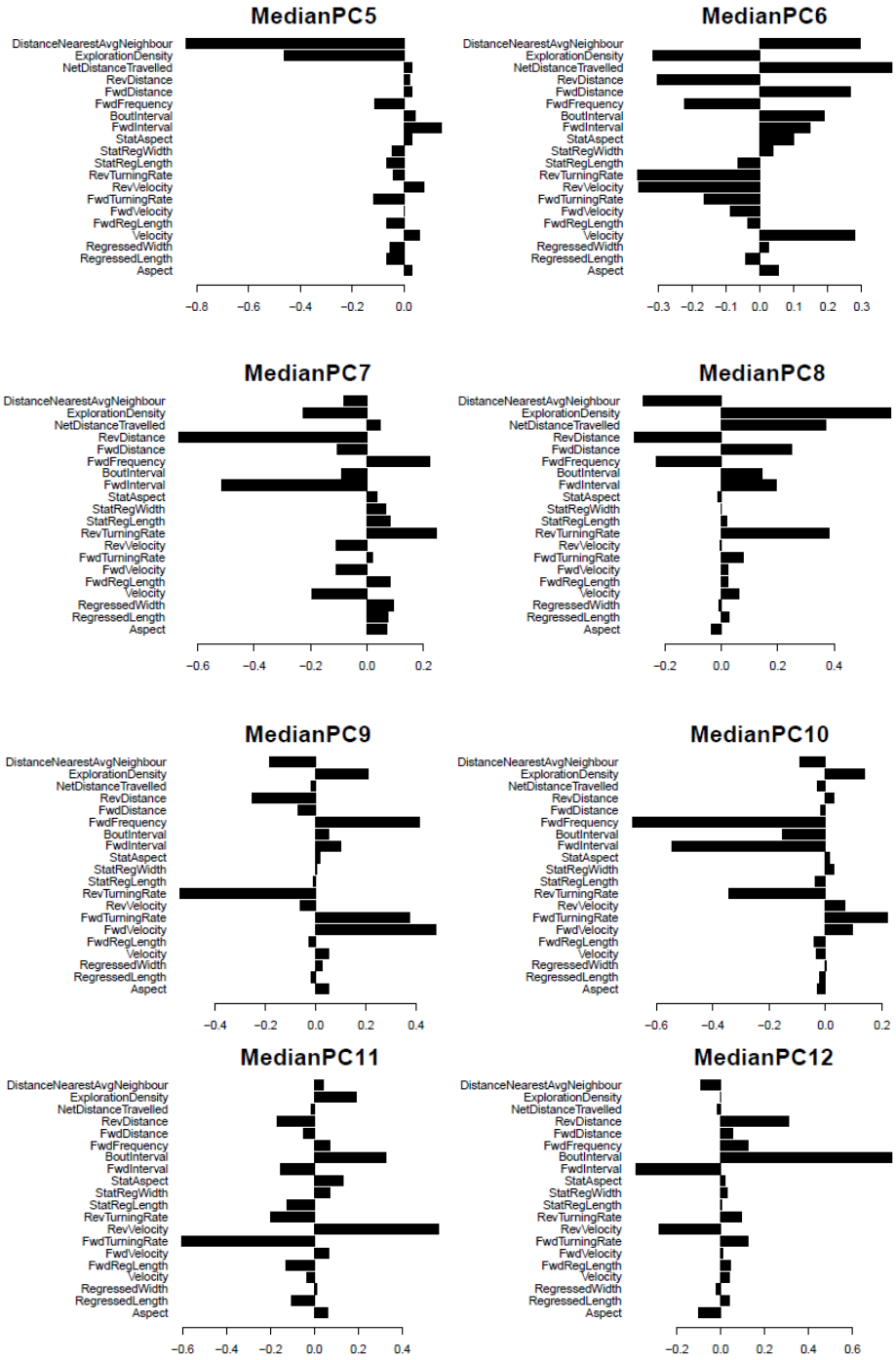
Feature (PC)	**B_{305}**	**Standard error**	***P***
MedianPC1	-0.39012739	0.107096559	0.0005*
	-0.24337848	0.081459599	0.005*
MedianPC2	0.20385294	0.065829214	0.01*
	0.18818060	0.057288055	0.005*
MedianPC3	-0.20980173	0.091994002	0.0275*
	-0.14807996	0.068544093	0.0325*
MedianPC4	-0.224250019	0.10109728	0.0345*
	-0.12757036	0.071699367	0.0825
MedianPC5	0.185499332	0.131024238	0.1125
MedianPC6	-0.008882142	0.152874121	0.476
MedianPC7	0.034870455	0.204833885	0.4385
MedianPC8	-0.390410707	0.377181091	0.2045
MedianPC9	2.439508	0.7738418	0.0045*
	2.00771858	0.690208715	0.005*
MedianPC10	-2.828047843	1.509129912	0.064
	-3.142029936	1.461882734	0.0335*
MADPC1	0.10965709	0.049227899	0.035*
	0.058371678	0.041409059	0.1085
MADPC2	-0.618269494	0.128800867	0*
	-0.42760172	0.095907186	0.0005*
MADPC3	0.093087478	0.068858329	0.1275
MADPC4	0.14021865	0.081794367	0.0735
	0.10680335	0.072862095	0.1045
MADPC5	-0.334485792	0.173972631	0.058
	-0.252686803	0.132459522	0.0565
MADPC6	-0.381924031	0.127050897	0.008*
	-0.255586436	0.100300283	0.0175*
MADPC7	0.261088873	0.109438142	0.0235*
	0.26892545	0.091018218	0.0065*
MADPC8	0.017430098	0.152641706	0.4645
MADPC9	0.210113819	0.156843511	0.1315
MADPC10	-0.372712149	0.183687594	0.054*
	-0.274861201	0.172461045	0.1095
MADPC11	0.163638997	0.265562389	0.3125
MADPC12	-0.372578262	0.361865843	0.2145
MADPC13	-1.160732509	0.414130548	0.0095*
	-0.892154323	0.380278773	0.031*

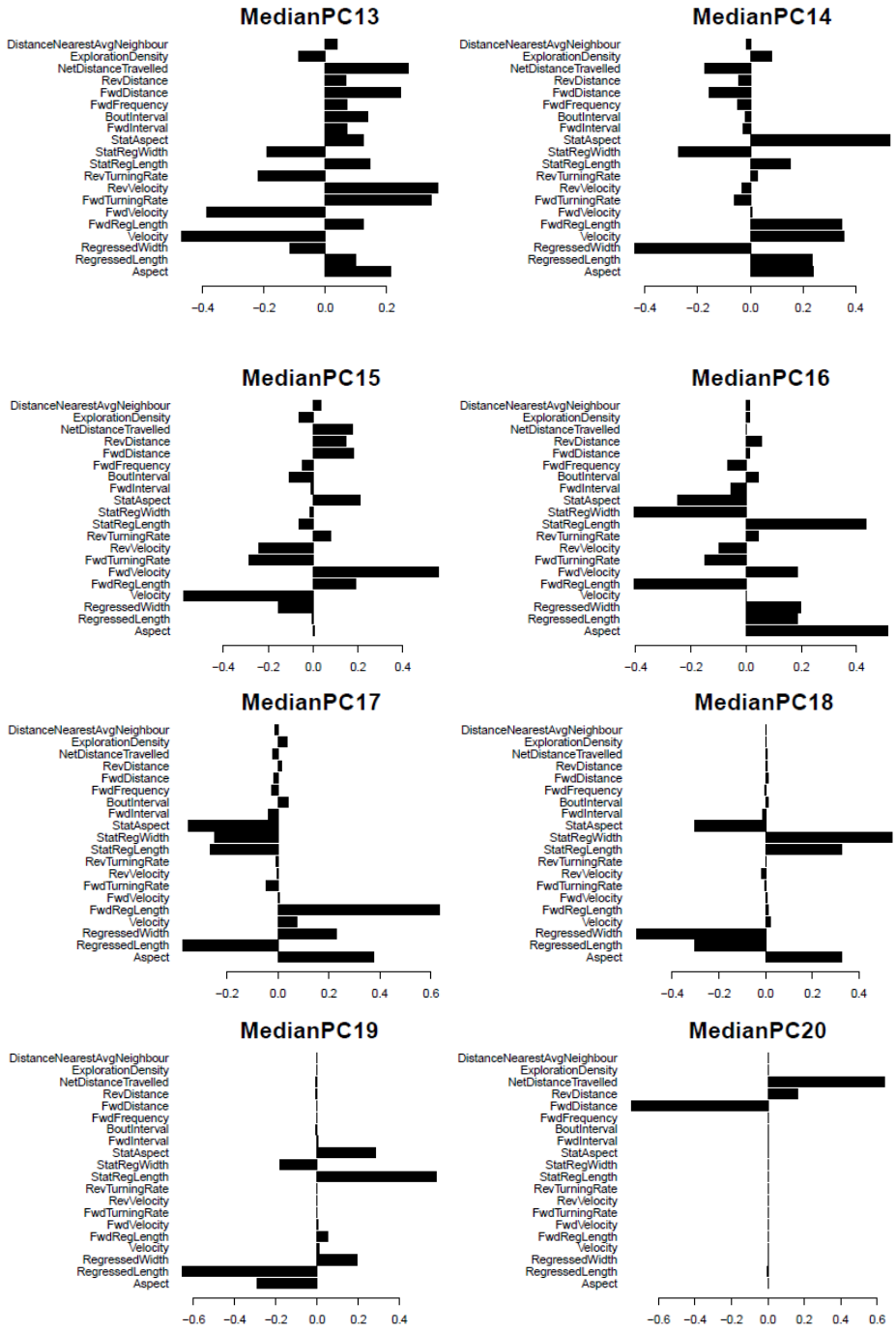
Appendix 4.5: Principal component loadings obtained from the separate principal components analyses on behaviour centrality and behaviour variability features.

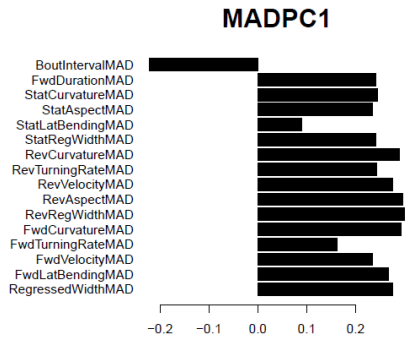
Here are the loadings for the principal components obtained in the multivariate selection surface using the principal components obtained from feature medians and MADs. These are the eigenvectors (v) described in Equation 2.9.

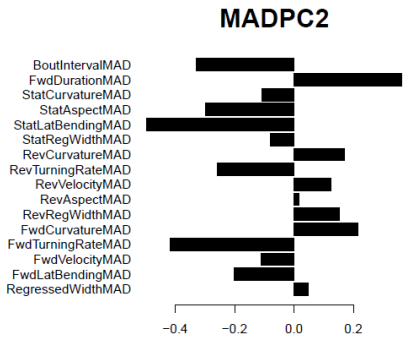
Appendix 4.5.1: Principal component loadings obtained from the behavioural features present in the multivariate selection surface in NaCl 25 mM.

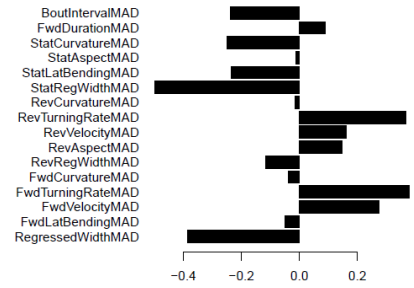


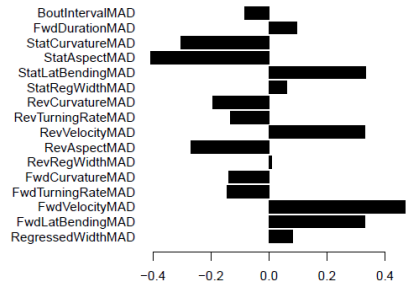

MedianPC5
DistanceNearestAvgNeighbour
ExplorationDensity
NetDistanceTravelled
RevDistance
FwdDistance
FwdFrequency
BoutInterval
FwdInterval
StatAspect
StatRegWidth
StatRegLength
RevTurningRate
RevVelocity
FwdTurningRate
FwdVelocity
FwdRegLength
Velocity
RegressedWidth
RegressedLength
Aspect
−0.8 −0.6 −0.4 −0.2 0.0
MedianPC6
−0.3 −0.2 −0.1 0.0 0.1 0.2 0.3
MedianPC7
−0.6 −0.4 −0.2 0.0 0.2
MedianPC8
−0.2 0.0 0.2 0.4
MedianPC9
−0.4 −0.2 0.0 0.2 0.4
MedianPC10
−0.6 −0.4 −0.2 0.0 0.2
MedianPC11
−0.6 −0.4 −0.2 0.0 0.2 0.4
MedianPC12
−0.2 0.0 0.2 0.4 0.6

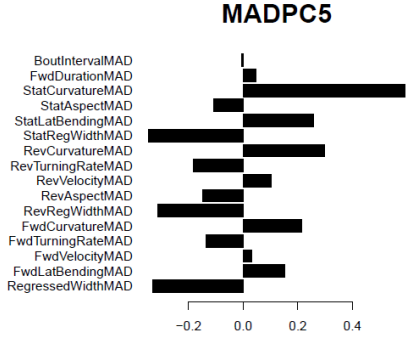

MedianPC13
DistanceNearestAvgNeighbour
ExplorationDensity
NetDistanceTravelled
RevDistance
FwdDistance
FwdFrequency
BoutInterval
FwdInterval
StatAspect
StatRegWidth
StatRegLength
RevTurningRate
RevVelocity
FwdTurningRate
FwdVelocity
FwdRegLength
Velocity
RegressedWidth
RegressedLength
Aspect
−0.4 −0.2 0.0 0.2
MedianPC14
−0.4 −0.2 0.0 0.2 0.4
MedianPC15
−0.4 −0.2 0.0 0.2 0.4
MedianPC16
−0.4 −0.2 0.0 0.2 0.4
MedianPC17
−0.2 0.0 0.2 0.4 0.6
MedianPC18
−0.4 −0.2 0.0 0.2 0.4
MedianPC19
−0.6 −0.4 −0.2 0.0 0.2 0.4
MedianPC20
−0.6 −0.4 −0.2 0.0 0.2 0.4 0.6

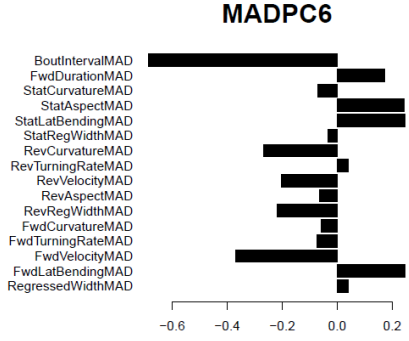

MADPC1
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.2
−0.1
0.0
0.1
0.2

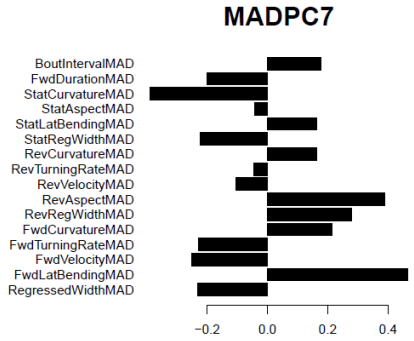

MADPC2
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2

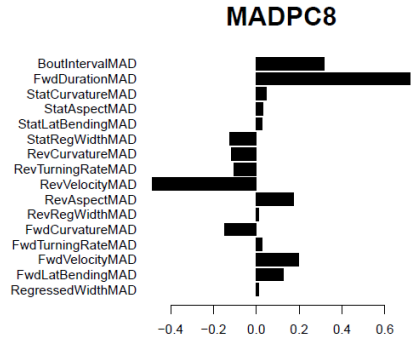

MADPC3
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2

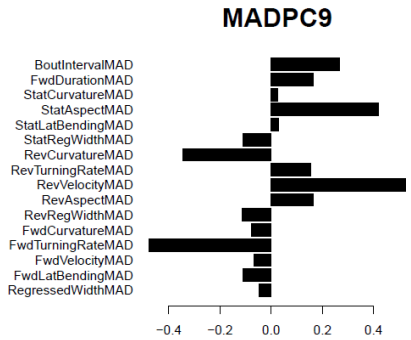

MADPC4
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2
0.4


MADPC5
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.2
0.0
0.2
0.4


MADPC6
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.6
−0.4
−0.2
0.0
0.2


MADPC7
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.2
0.0
0.2
0.4


MADPC8
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2
0.4
0.6


MADPC9
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
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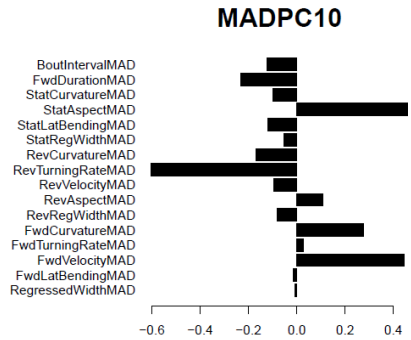
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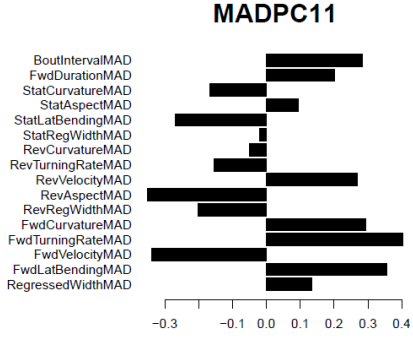
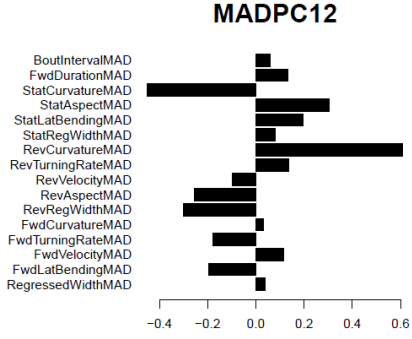
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.6 −0.4 −0.2 0.0 0.2 0.4


MADPC11
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.3 −0.1 0.0 0.1 0.2 0.3 0.4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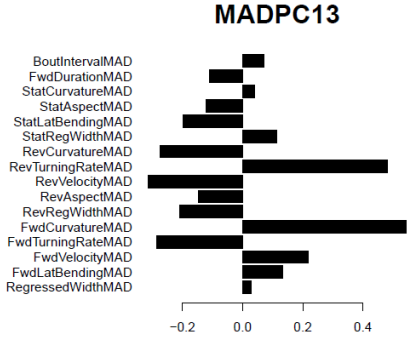
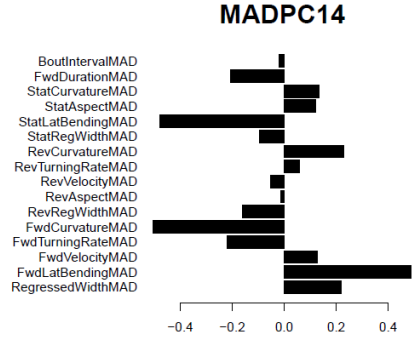
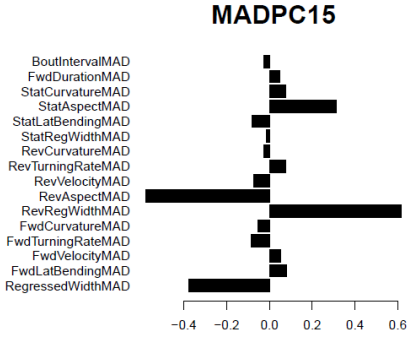
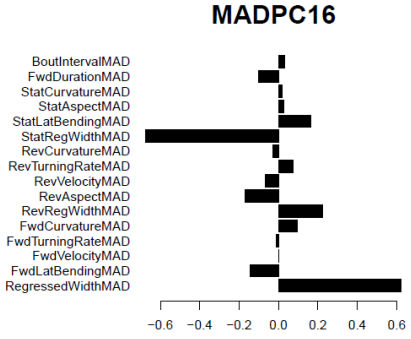
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4 −0.2 0.0 0.2 0.4 0.6

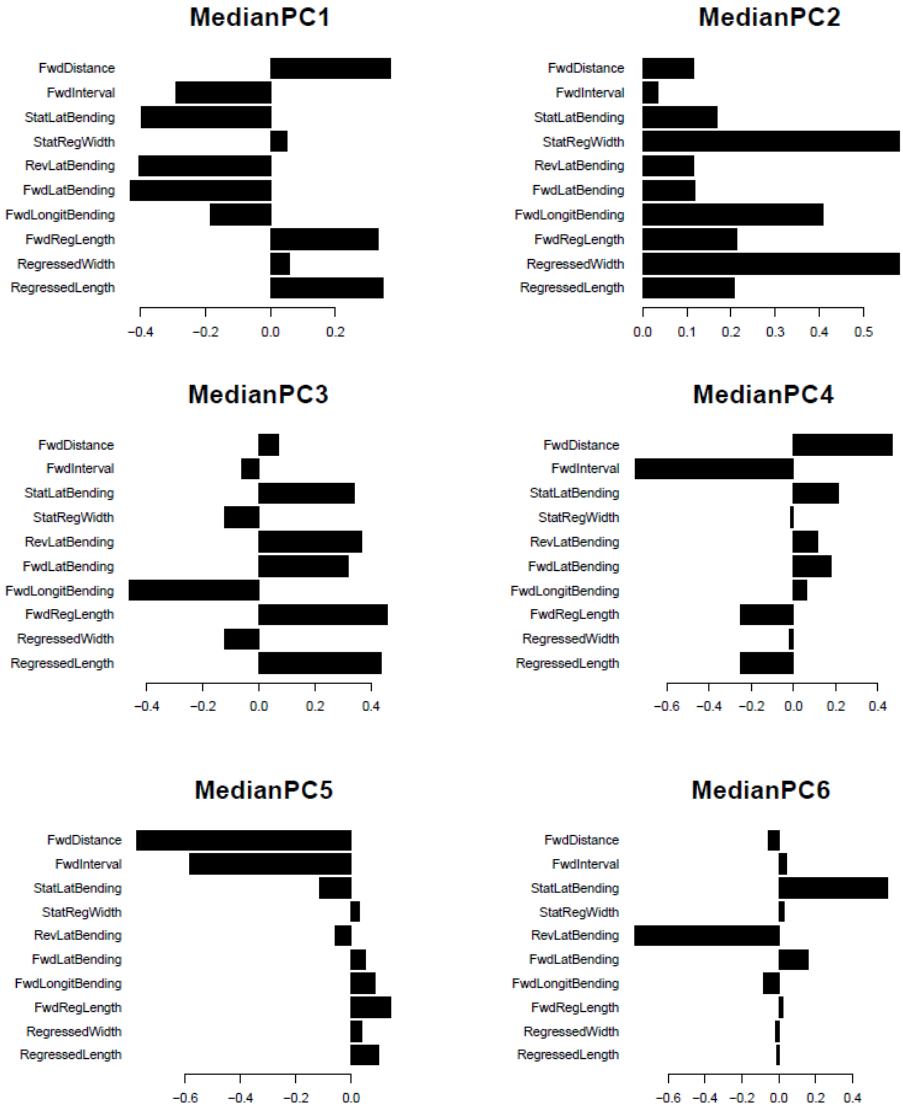

MADPC13
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.2 0.0 0.2 0.4

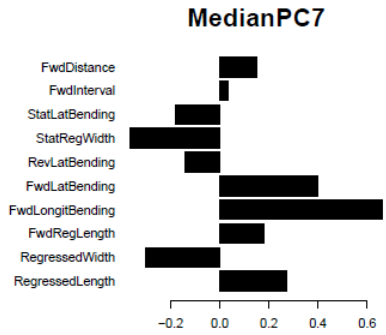

MADPC14
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4 −0.2 0.0 0.2 0.4

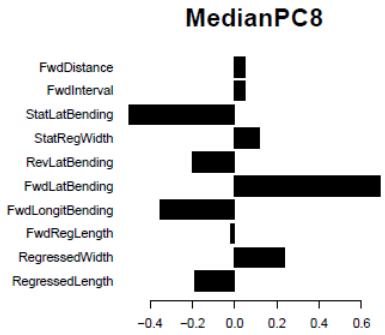

MADPC15
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.4 −0.2 0.0 0.2 0.4 0.6

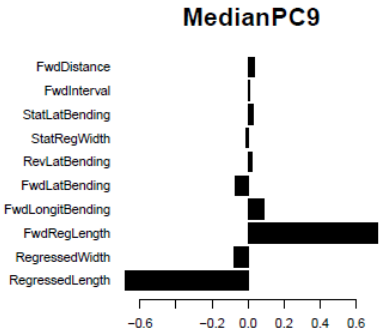

MADPC16
BoutIntervalMAD
FwdDurationMAD
StatCurvatureMAD
StatAspectMAD
StatLatBendingMAD
StatRegWidthMAD
RevCurvatureMAD
RevTurningRateMAD
RevVelocityMAD
RevAspectMAD
RevRegWidthMAD
FwdCurvatureMAD
FwdTurningRateMAD
FwdVelocityMAD
FwdLatBendingMAD
RegressedWidthMAD
−0.6 −0.4 −0.2 0.0 0.2 0.4 0.6

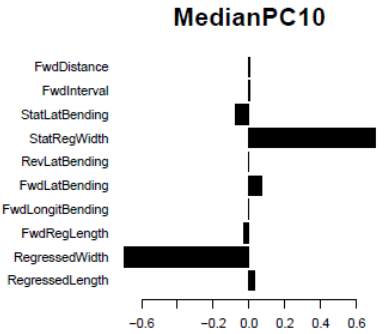
Appendix 4.5.2: Principal component loadings obtained from the behavioural features present in the multivariate selection surface in NaCl 305 mM.

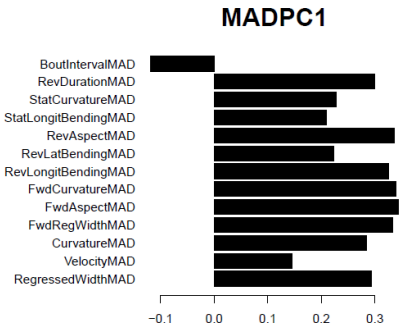


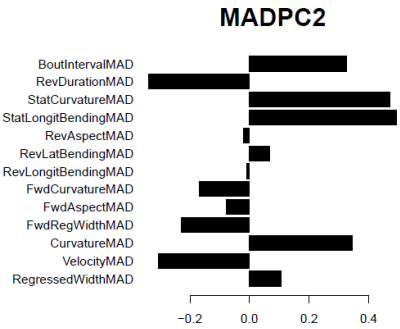

MedianPC7
FwdDistance
FwdInterval
StatLatBending
StatRegWidth
RevLatBending
FwdLatBending
FwdLongitBending
FwdRegLength
RegressedWidth
RegressedLength
−0.2
0.0
0.2
0.4
0.6

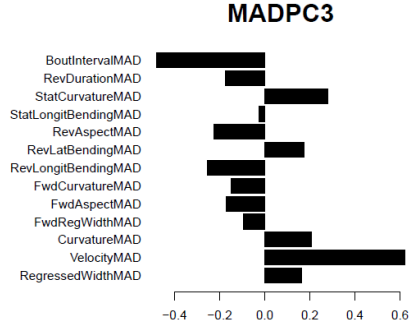

MedianPC8
FwdDistance
FwdInterval
StatLatBending
StatRegWidth
RevLatBending
FwdLatBending
FwdLongitBending
FwdRegLength
RegressedWidth
RegressedLength
−0.4
−0.2
0.0
0.2
0.4
0.6

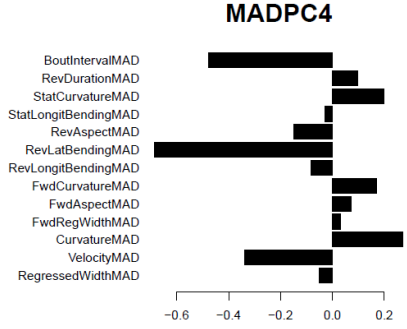

MedianPC9
FwdDistance
FwdInterval
StatLatBending
StatRegWidth
RevLatBending
FwdLatBending
FwdLongitBending
FwdRegLength
RegressedWidth
RegressedLength
−0.6
−0.2
0.0
0.2
0.4
0.6

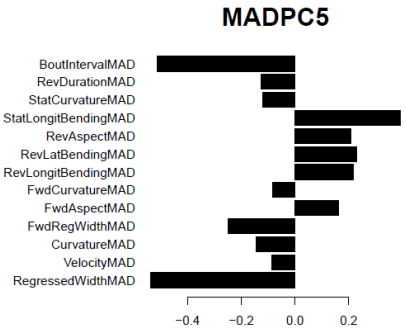

MedianPC10
FwdDistance
FwdInterval
StatLatBending
StatRegWidth
RevLatBending
FwdLatBending
FwdLongitBending
FwdRegLength
RegressedWidth
RegressedLength
−0.6
−0.2
0.0
0.2
0.4
0.6

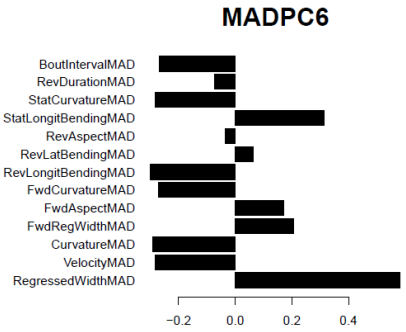

MADPC1
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.1
0.0
0.1
0.2
0.3

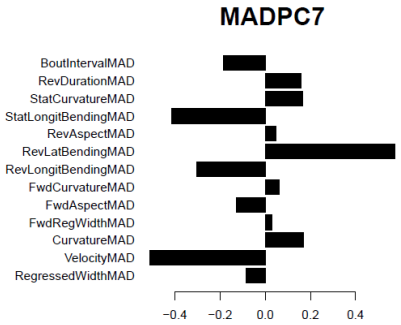

MADPC2
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.2
0.0
0.2
0.4

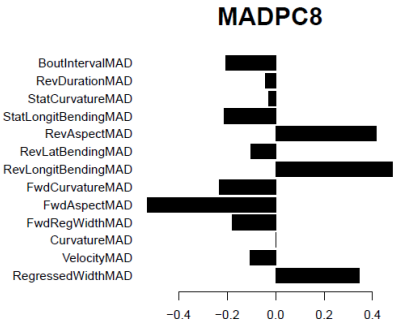

MADPC3
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2
0.4
0.6

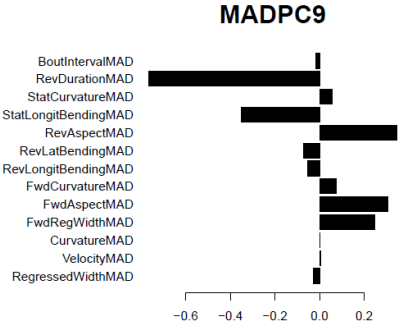

MADPC4
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.6
−0.4
−0.2
0.0
0.2

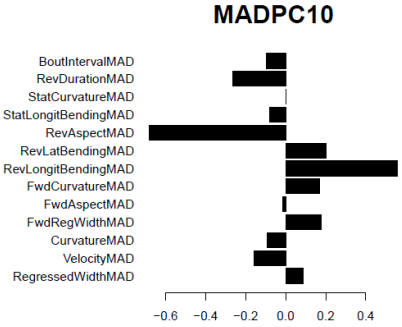

MADPC5
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2

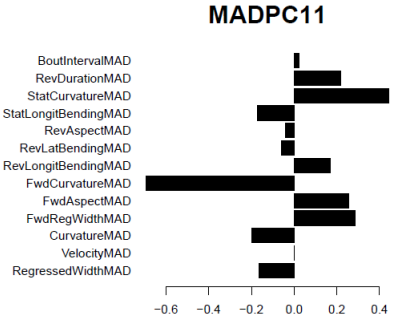

MADPC6
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.2
0.0
0.2
0.4

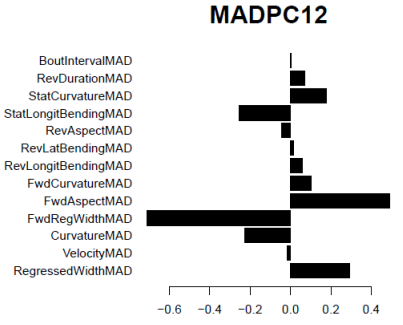

MADPC7
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2
0.4

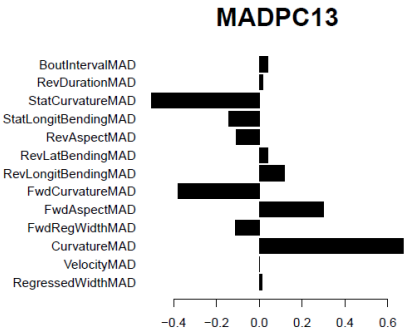

MADPC8
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.4
−0.2
0.0
0.2
0.4


MADPC9
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.6
−0.4
−0.2
0.0
0.2


MADPC10
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.6
−0.4
−0.2
0.0
0.2
0.4


MADPC11
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.6
−0.4
−0.2
0.0
0.2
0.4

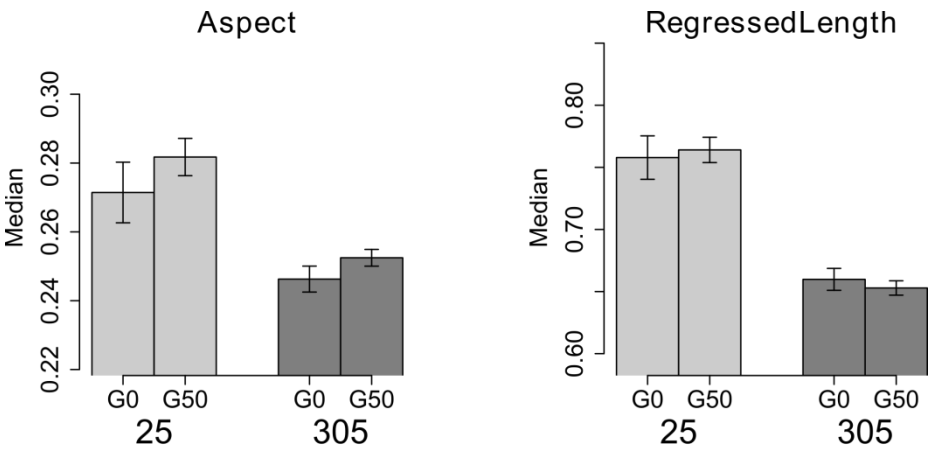

MADPC12
BoutIntervalMAD
RevDurationMAD
StatCurvatureMAD
StatLongitBendingMAD
RevAspectMAD
RevLatBendingMAD
RevLongitBendingMAD
FwdCurvatureMAD
FwdAspectMAD
FwdRegWidthMAD
CurvatureMAD
VelocityMAD
RegressedWidthMAD
−0.6
−0.4
−0.2
0.0
0.2
0.4

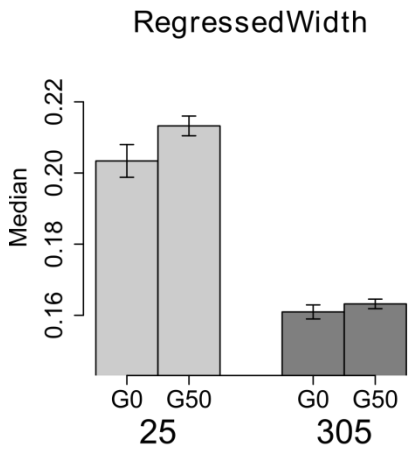


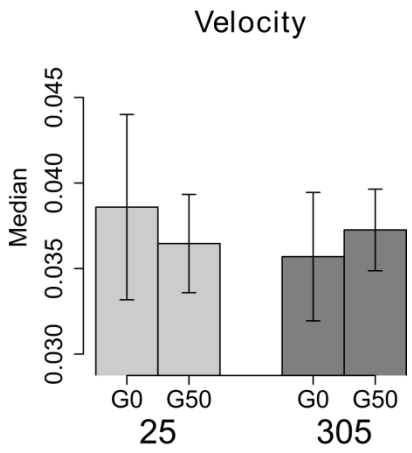
Appendix 5: Evolution of behaviour feature centrality.

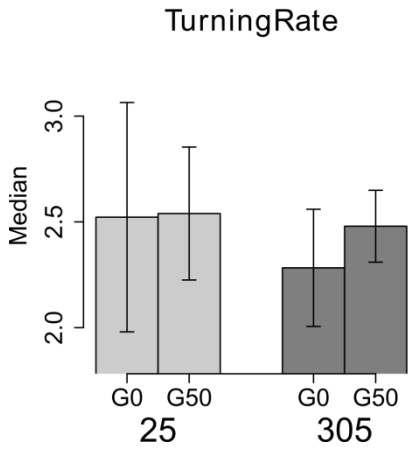
Below are the estimates given by the linear mixed models applied to each feature median and their respective standard errors for each of generations 0 and 50. Numbers in the x-axis represent the environments, defined by NaCl concentration. Asterisks highlight the statistical significant responses under a significance level of 0.05.

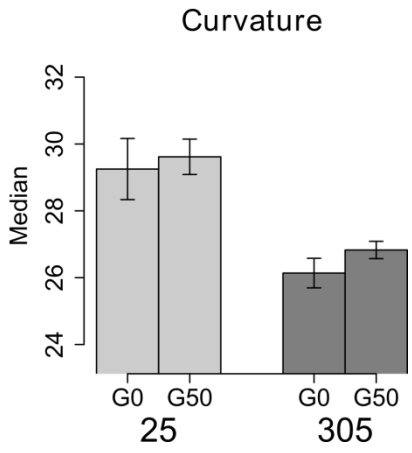
Appendix 5.1: Evolution of each behavioural feature median in the populations.

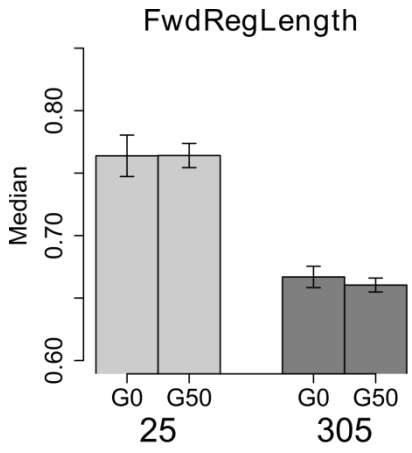


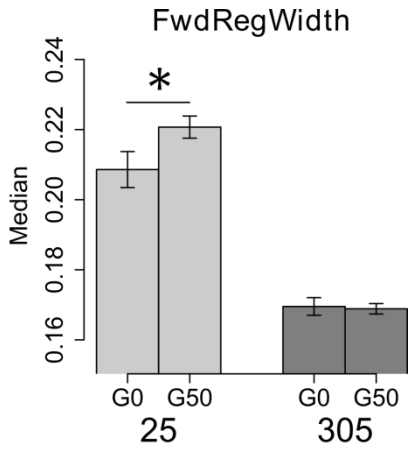

RegressedWidth
Median
0.16
0.18
0.20
0.22
G0
G50
G0
G50
25
305

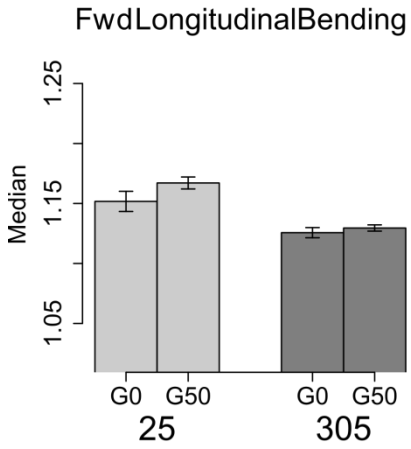

Velocity
Median
0.030
0.035
0.040
0.045
G0
G50
G0
G50
25
305

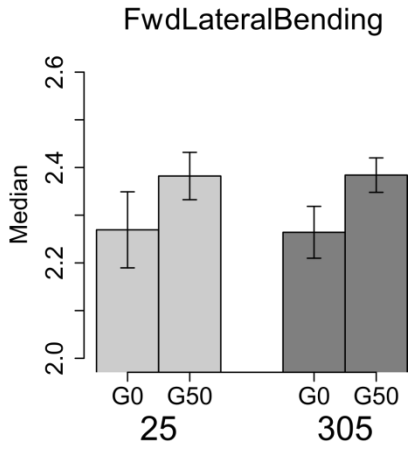

TurningRate
Median
2.0
2.5
3.0
G0
G50
G0
G50
25
305

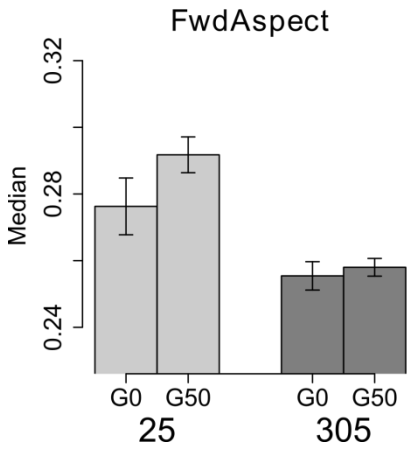

Curvature
Median
24
26
28
30
32
G0
G50
G0
G50
25
305


FwdRegLength
Median
0.60
0.70
0.80
G0
G50
G0
G50
25
305


FwdRegWidth
*
Median
0.16
0.18
0.20
0.22
0.24
G0
G50
G0
G50
25
305


FwdLongitudinalBending
Median
1.05
1.15
1.25
G0
G50
G0
G50
25
305


FwdLateralBending
Median
2.0
2.2
2.4
2.6
G0
G50
G0
G50
25
305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Median
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0.28
0.32
G0
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G0
G50
25
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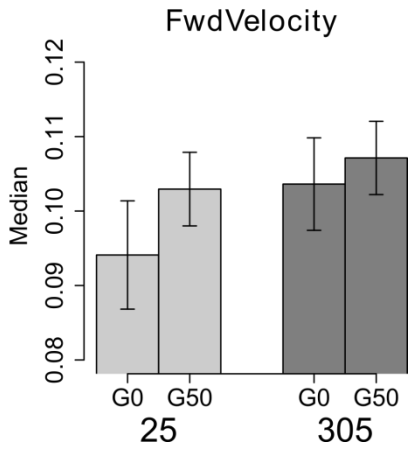
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Median
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0.11
0.12
G0
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G0
G50
25
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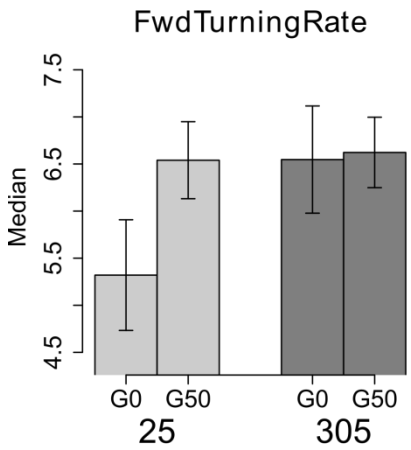
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5.5
6.5
7.5
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G50
G0
G50
25
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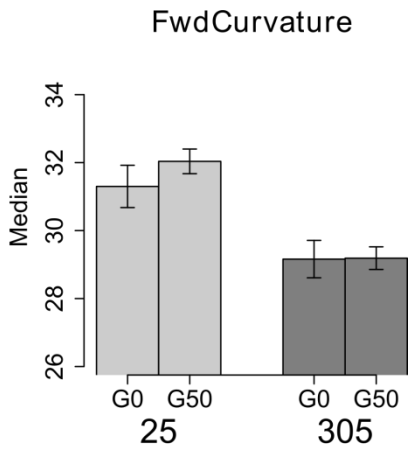
FwdCurvature
Median
26
28
30
32
34
G0
G50
G0
G50
25
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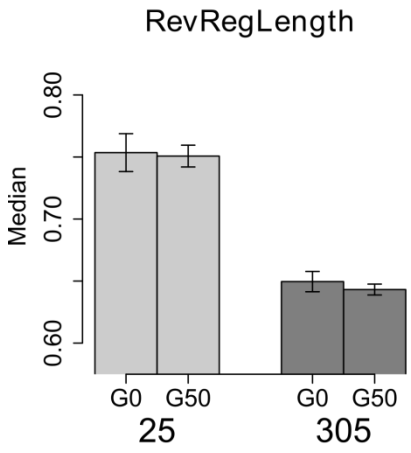
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Median
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0.70
0.80
G0
G50
G0
G50
25
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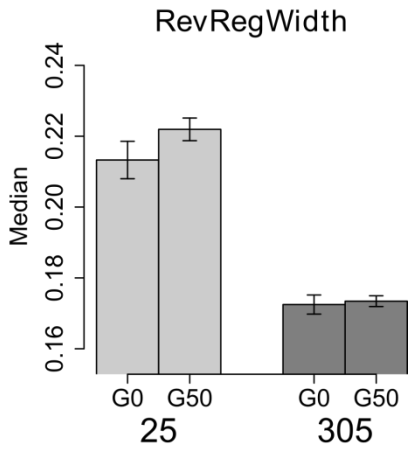
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Median
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0.20
0.22
0.24
G0
G50
G0
G50
25
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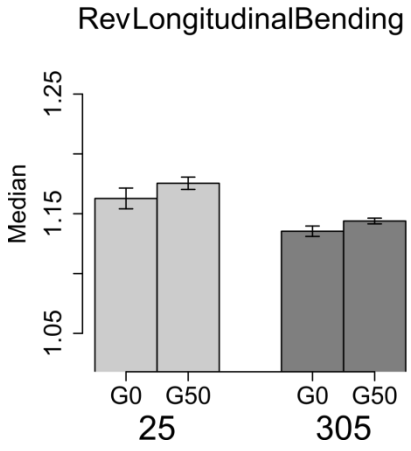
RevLongitudinalBending
Median
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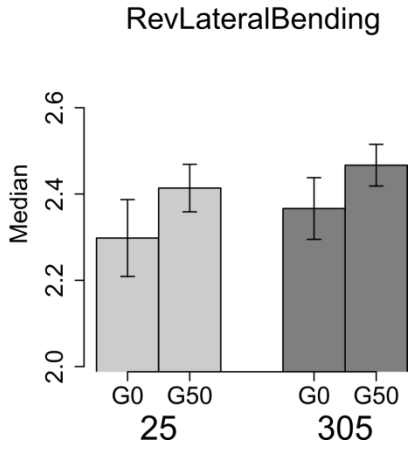
1.15
1.25
G0
G50
G0
G50
25
305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Median
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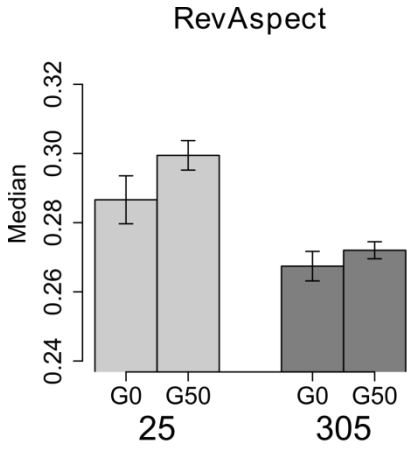
2.4
2.6
G0
G50
G0
G50
25
305


RevAspect
Median
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0.28
0.30
0.32
G0
G50
G0
G50
25
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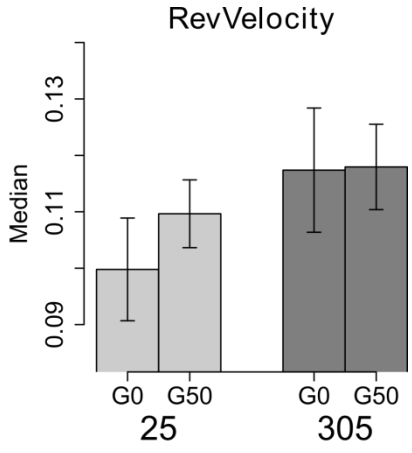
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Median
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0.11
0.13
G0
G50
G0
G50
25
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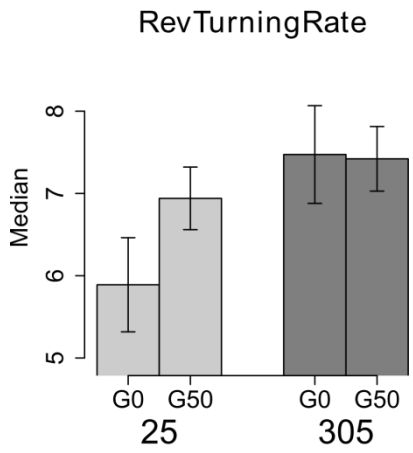
RevTurningRate
Median
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6
7
8
G0
G50
G0
G50
25
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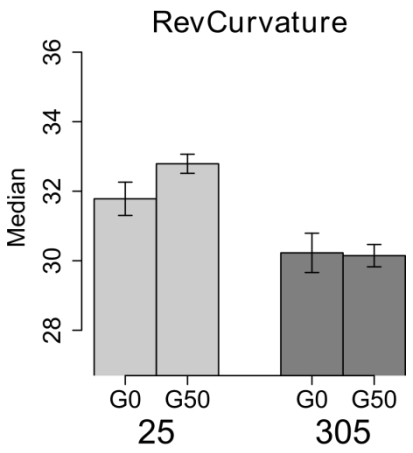
RevCurvature
Median
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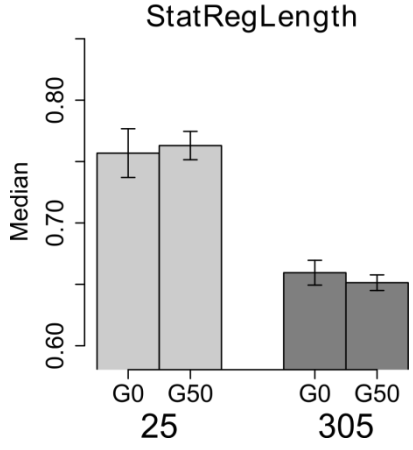
30
32
34
36
G0
G50
G0
G50
25
305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Median
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0.70
0.80
G0
G50
G0
G50
25
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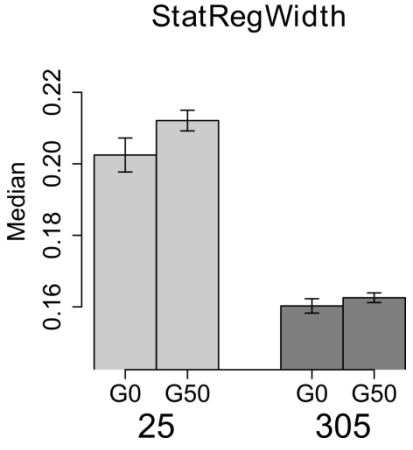
StatRegWidth
Median
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0.18
0.20
0.22
G0
G50
G0
G50
25
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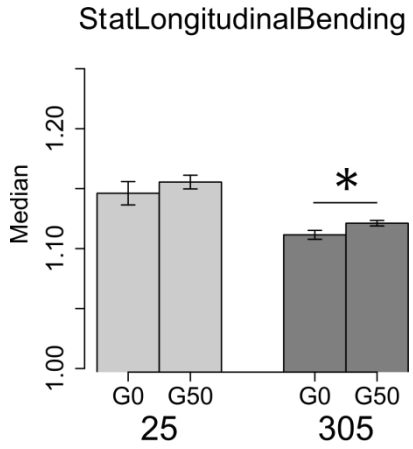
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Median
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1.20
*
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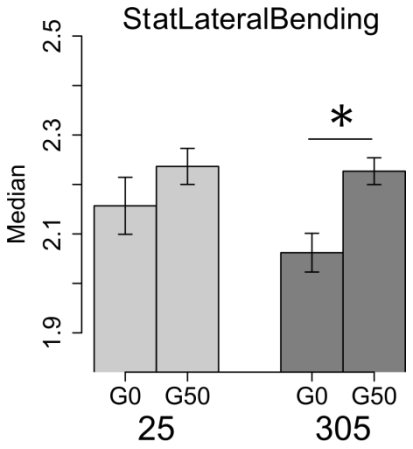
G50
G0
G50
25
305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Median
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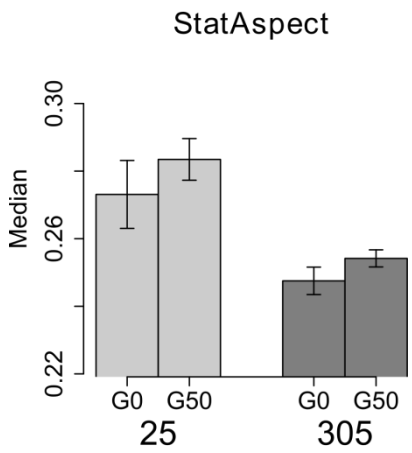
2.3
2.5
*
G0
G50
G0
G50
25
305


StatAspect
Median
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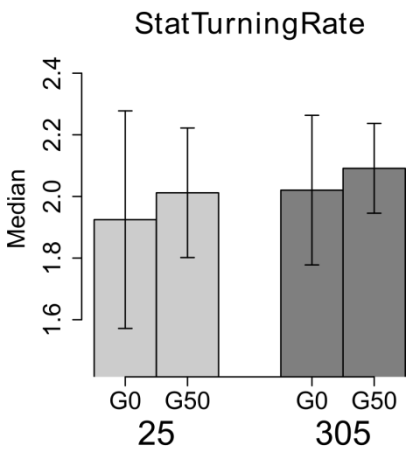
0.26
0.30
G0
G50
G0
G50
25
305


StatTurningRate
Median
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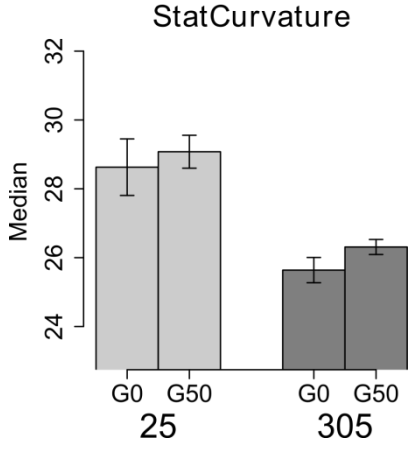
2.0
2.2
2.4
G0
G50
G0
G50
25
305


StatCurvature
Median
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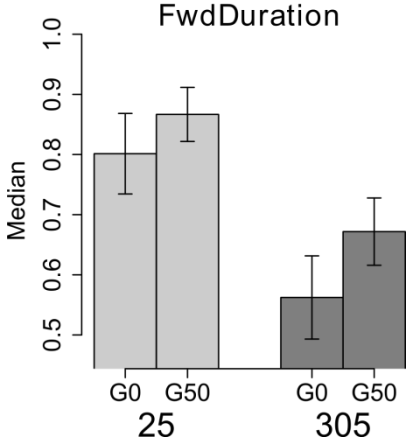
26
28
30
32
G0
G50
G0
G50
25
305


FwdDuration
Median
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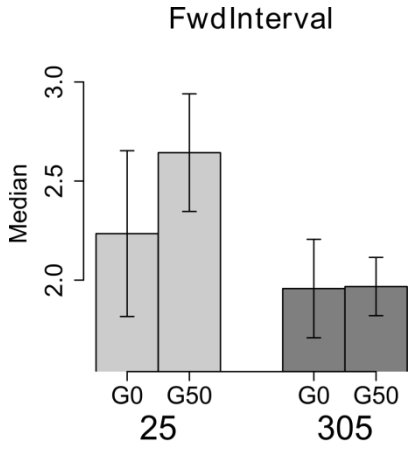
0.6
0.7
0.8
0.9
1.0
G0
G50
G0
G50
25
305


FwdInterval
Median
2.0
2.5
3.0
G0
G50
G0
G50
25
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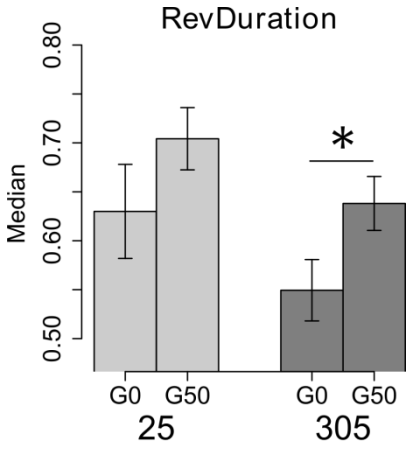
RevDuration
Median
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0.80
*
G0
G50
G0
G50
25
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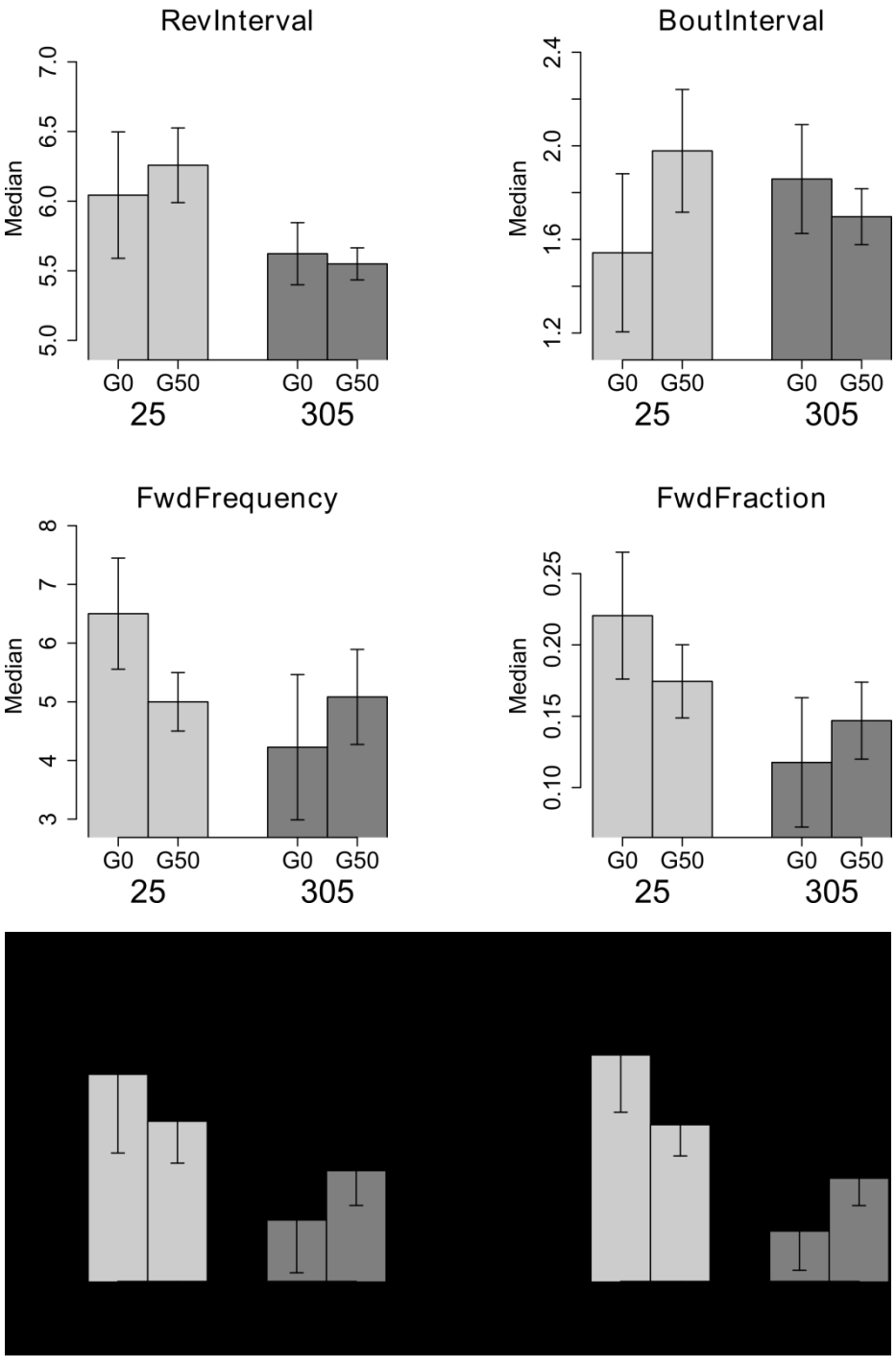
RevInterval
Median
5.0
5.5
6.0
6.5
7.0
G0
G50
G0
G50
25
305
BoutInterval
Median
1.2
1.6
2.0
2.4
G0
G50
G0
G50
25
305
FwdFrequency
Median
3
4
5
6
7
8
G0
G50
G0
G50
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Median
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0.15
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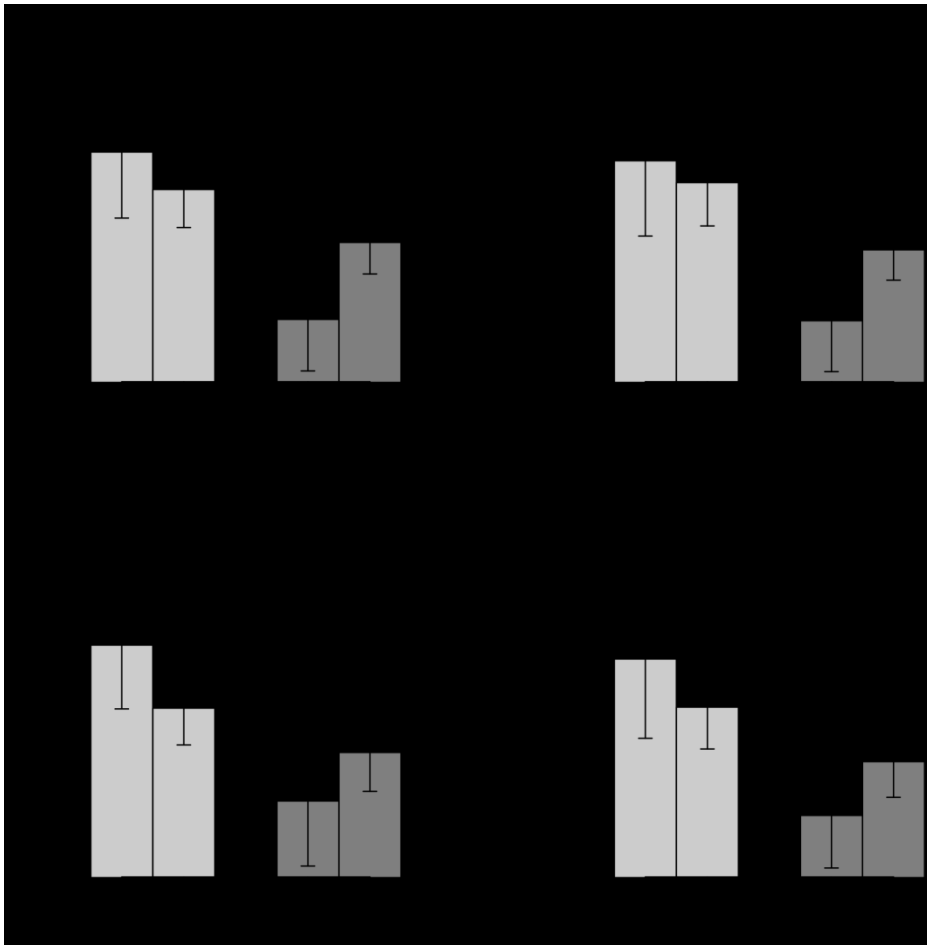
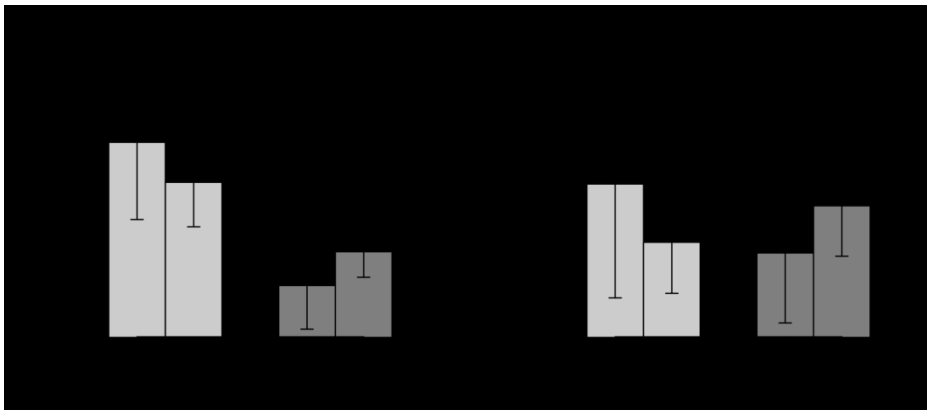
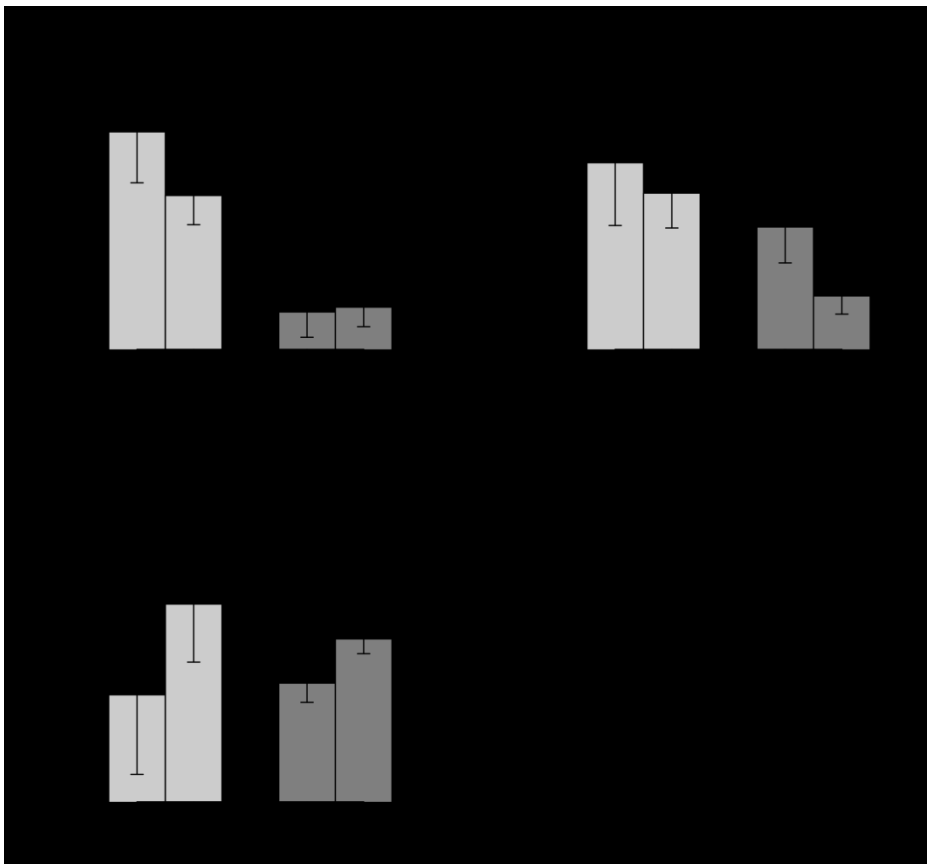
G0
G50
G0
G50
25
305

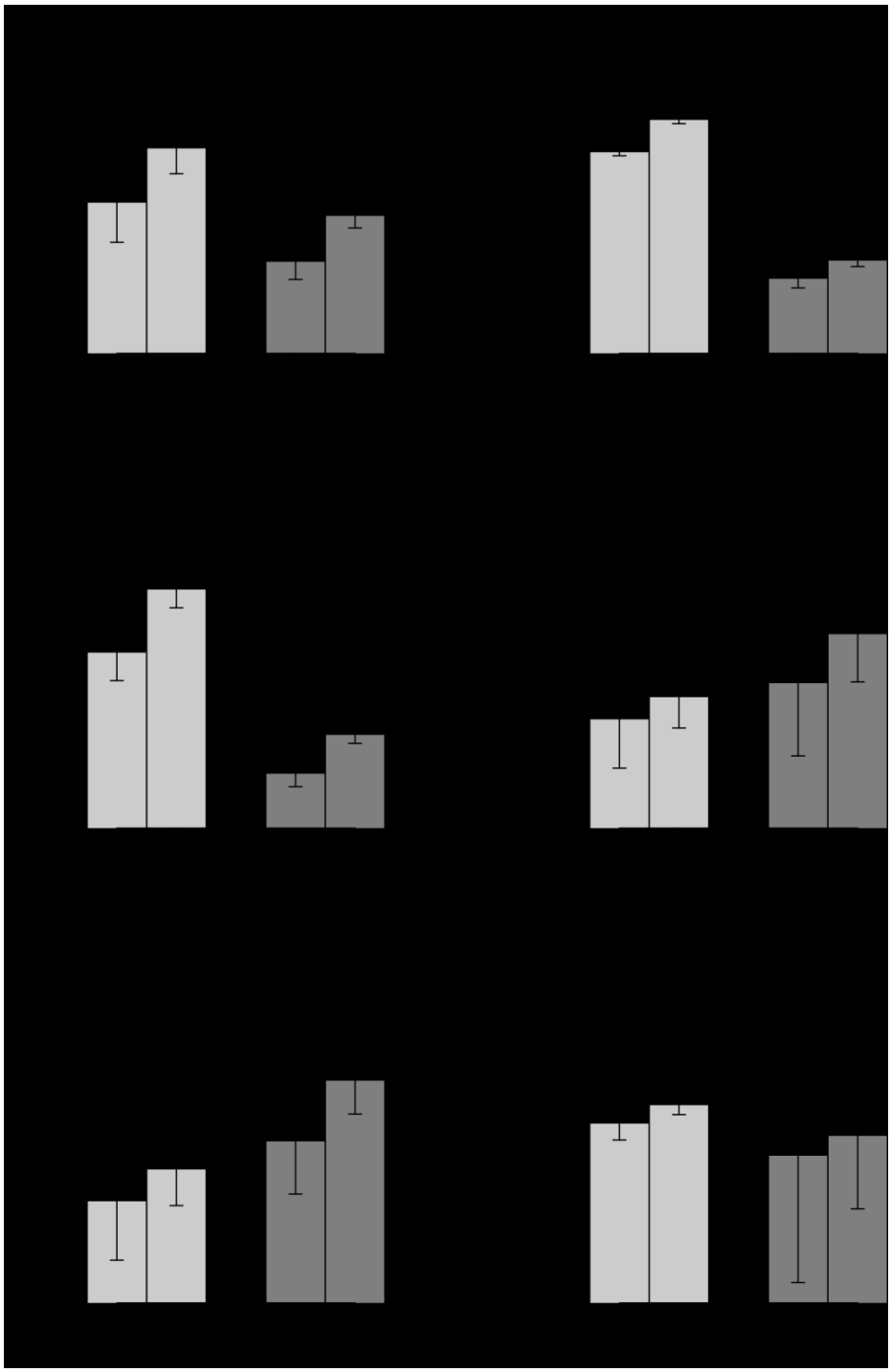


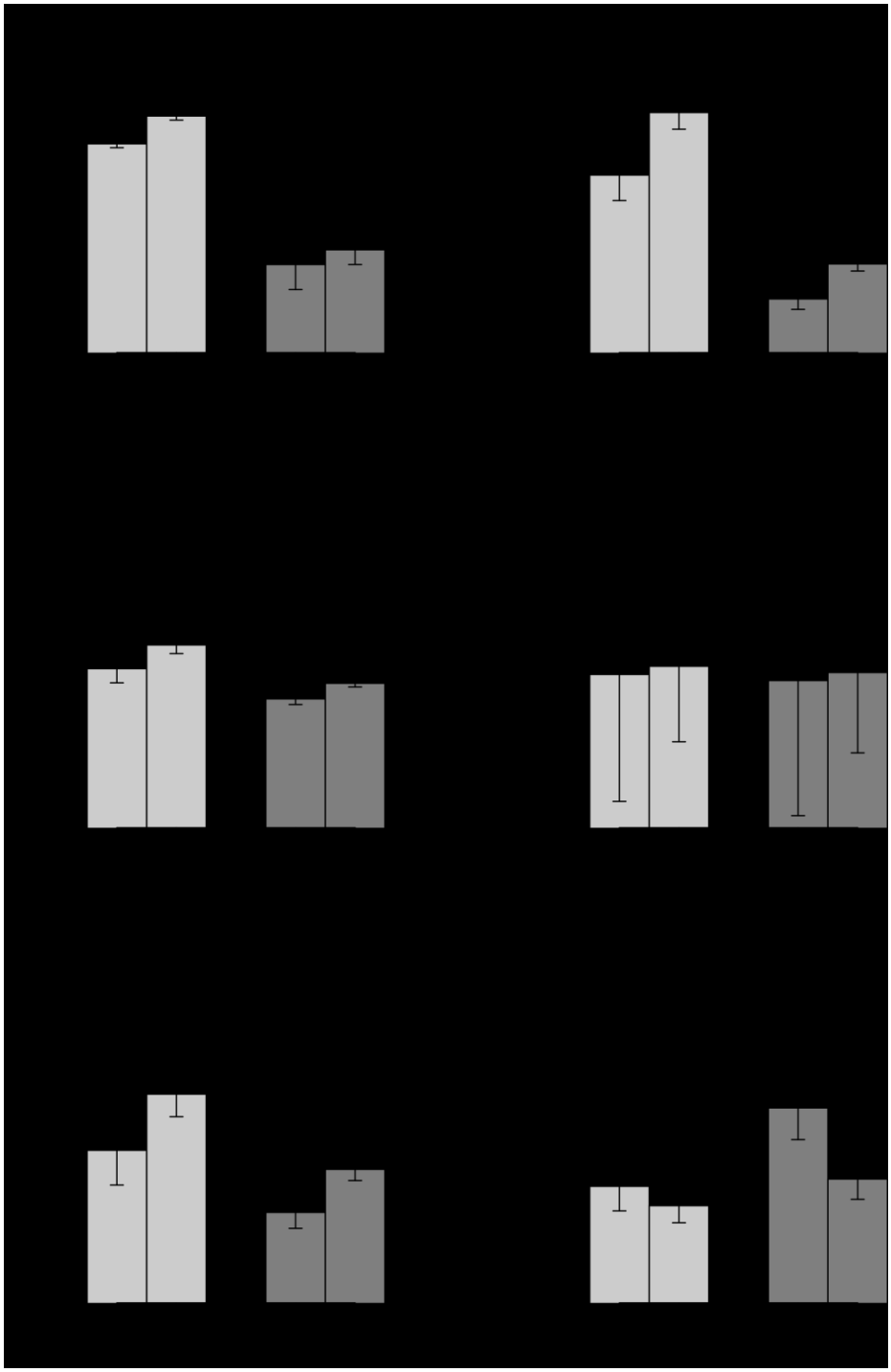


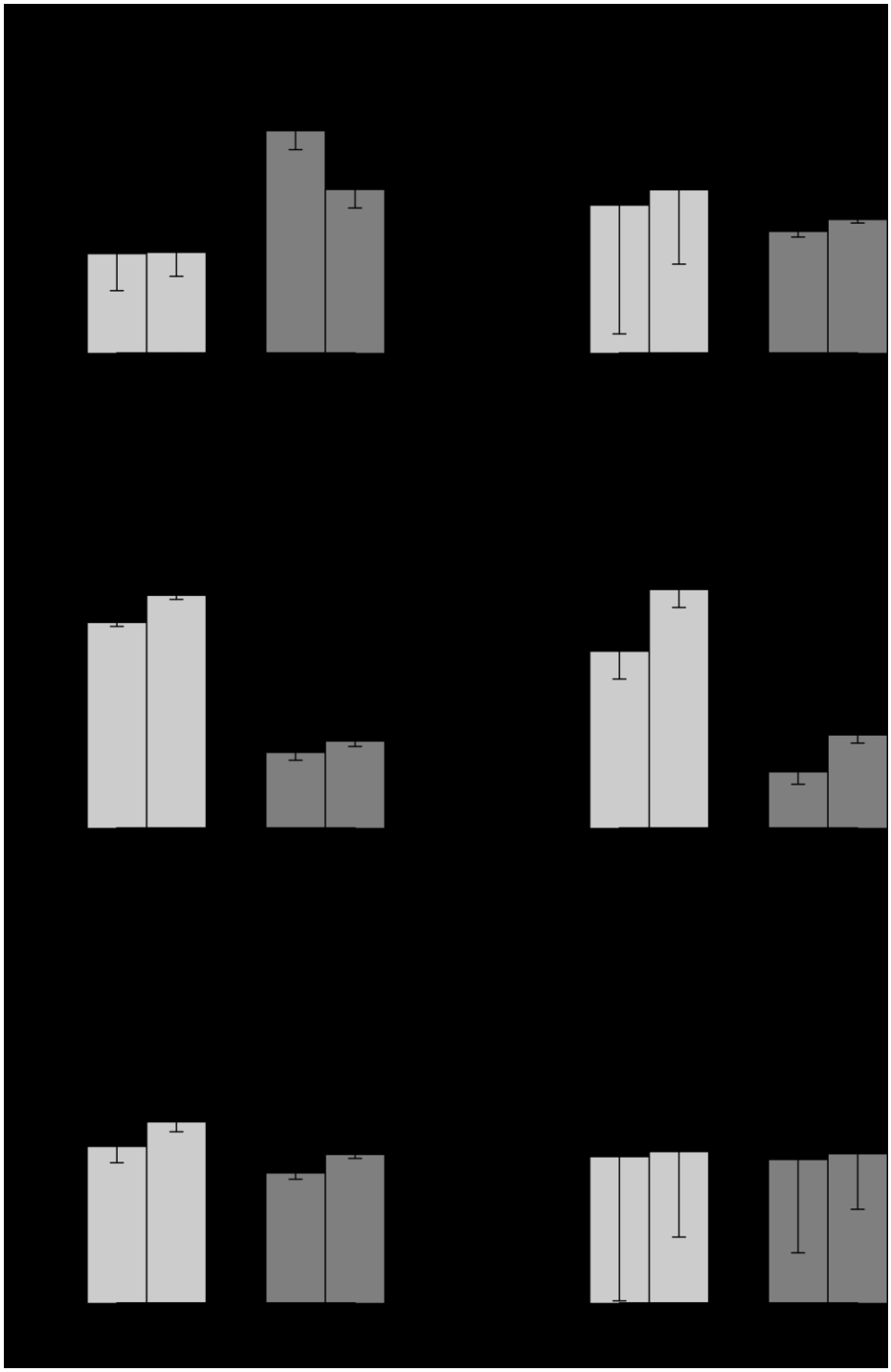


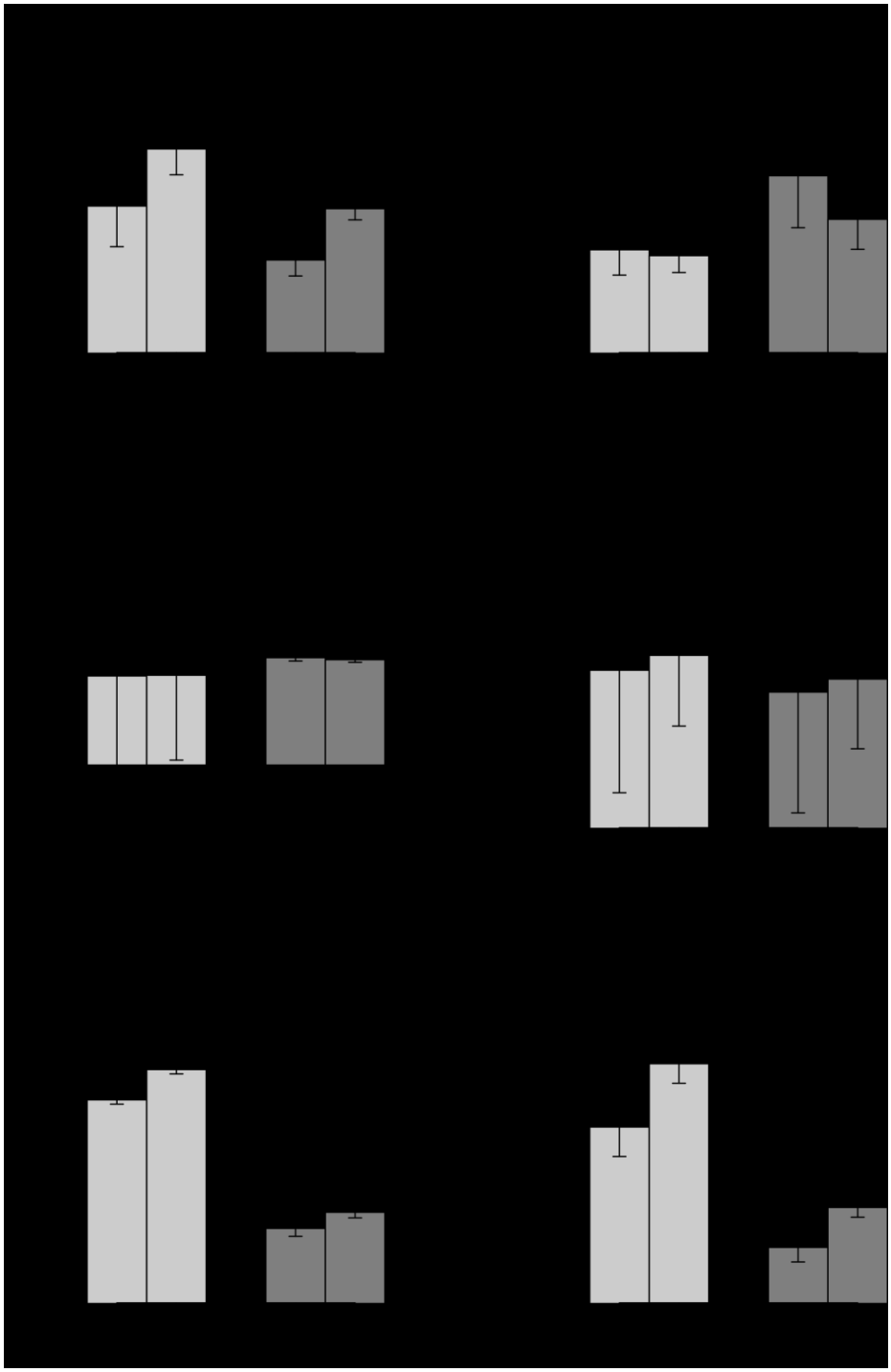
Appendix 5.2: Evolution of each behavioural feature median observed in the inbred lines.

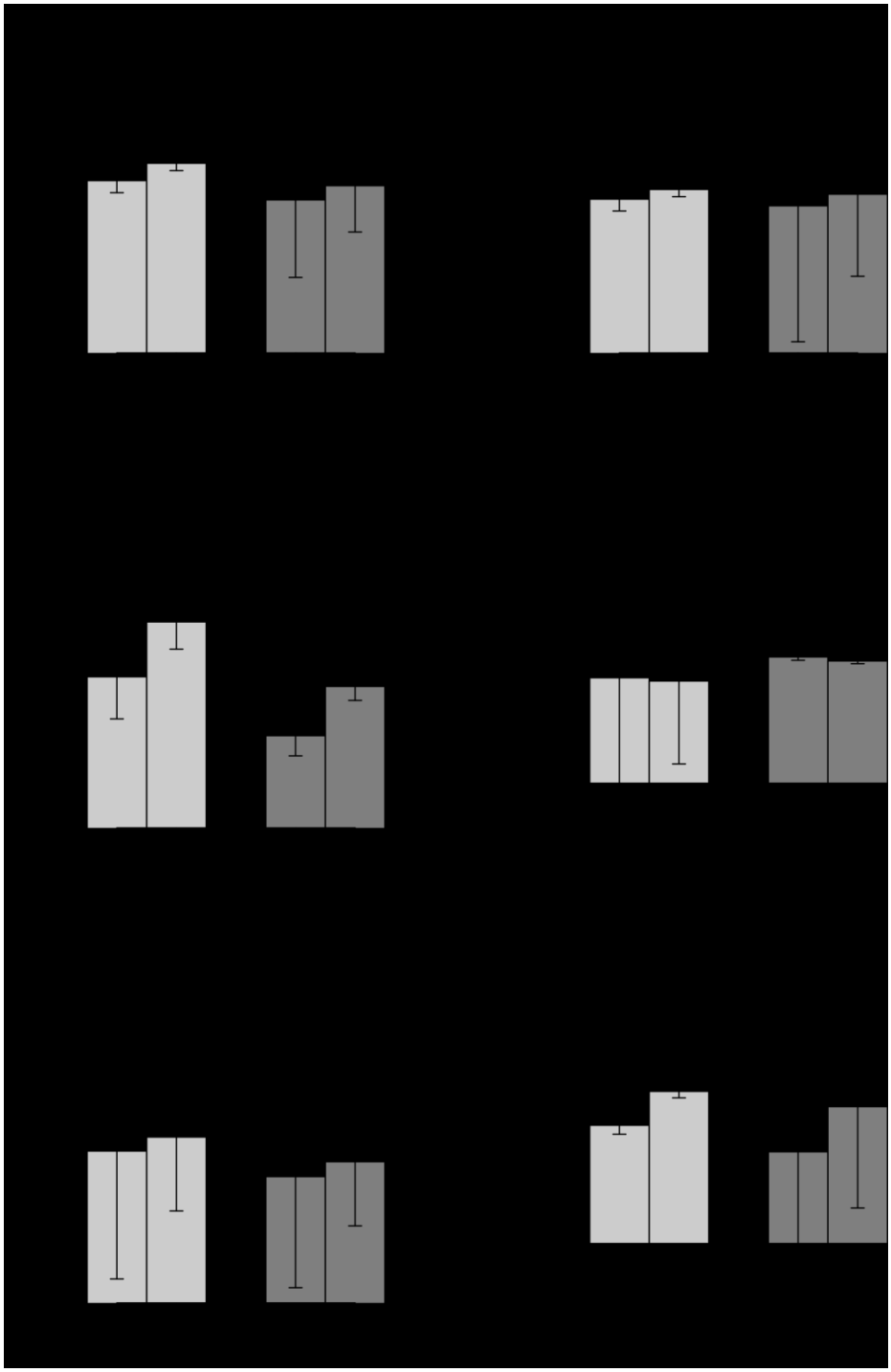
Some of the least-squares estimates here shown are associated with disproportionate standard errors, even higher than the estimates themselves. The reasons accounting for this phenomenon are not clear, yet the models when applied to these features have always shown a disproportionately high variance component in the population factor (not shown). There could be real heterogeneity among populations, yet little changes in the dataset lead to gross changes in the estimation of these standard errors. Therefore, I suggest either there is multicollinerity among the random effects (Hodges, 2013) – which seems likely given the highly complex, hierarchical model used (Equation 2.13).

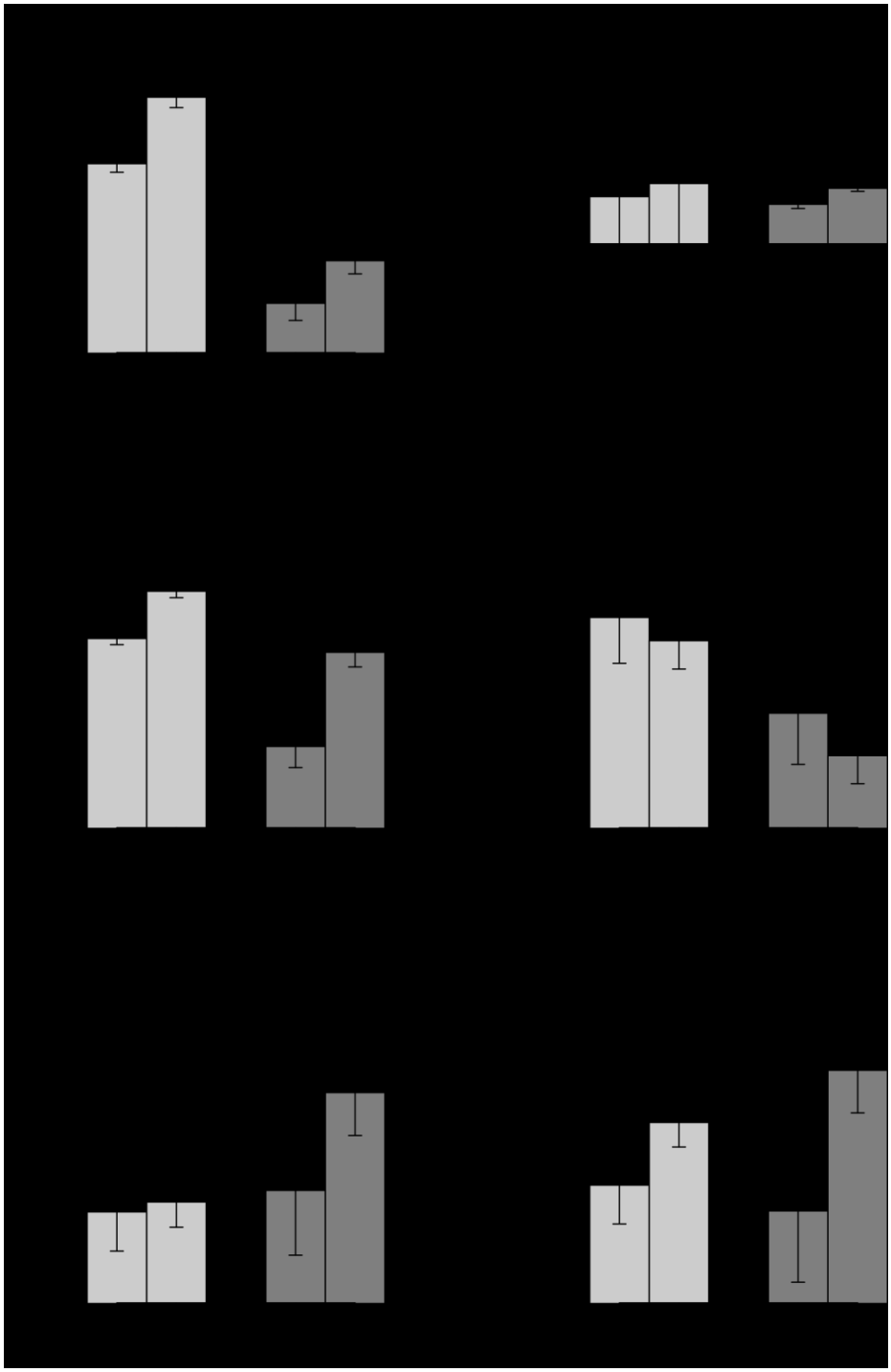


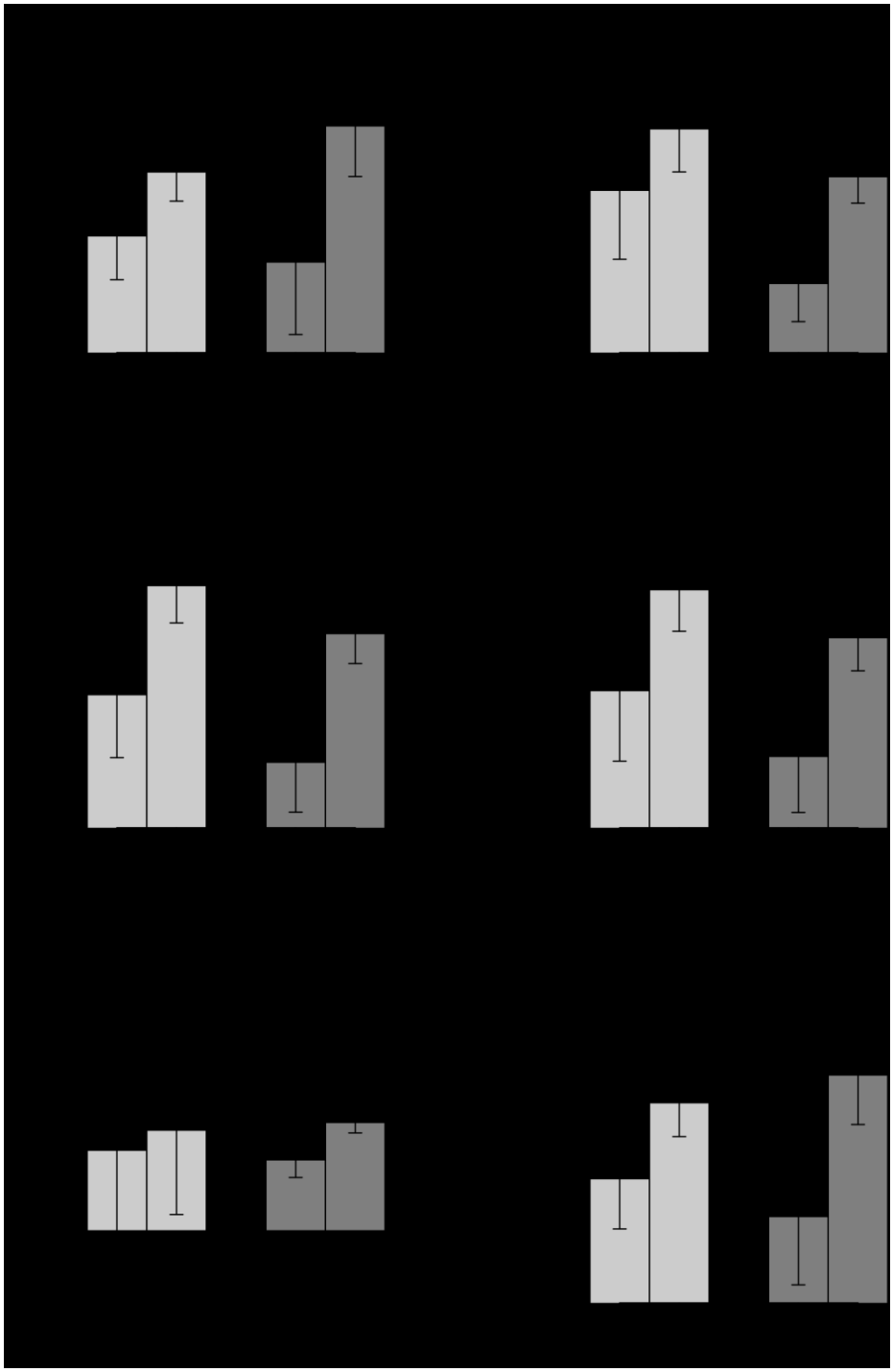


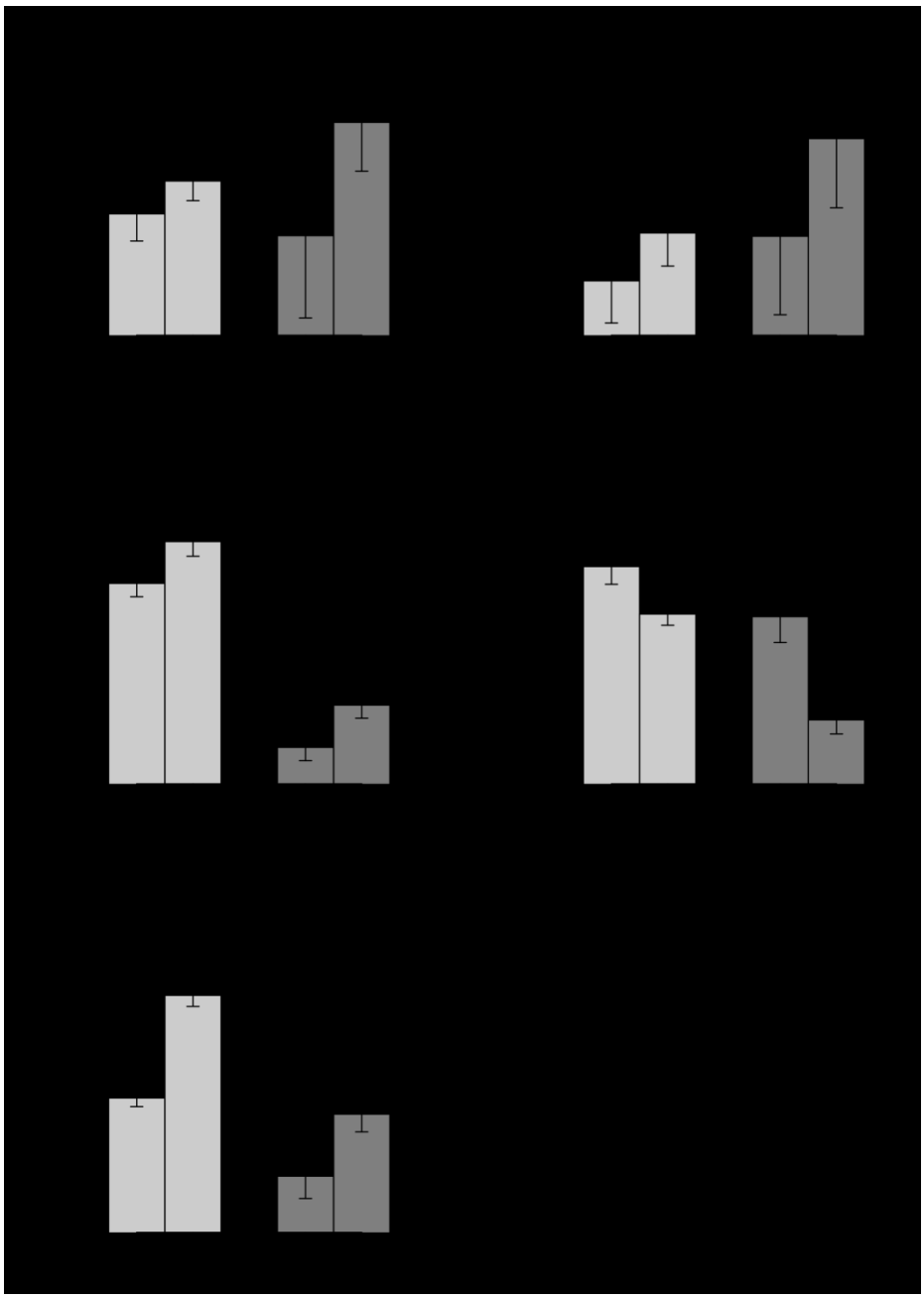












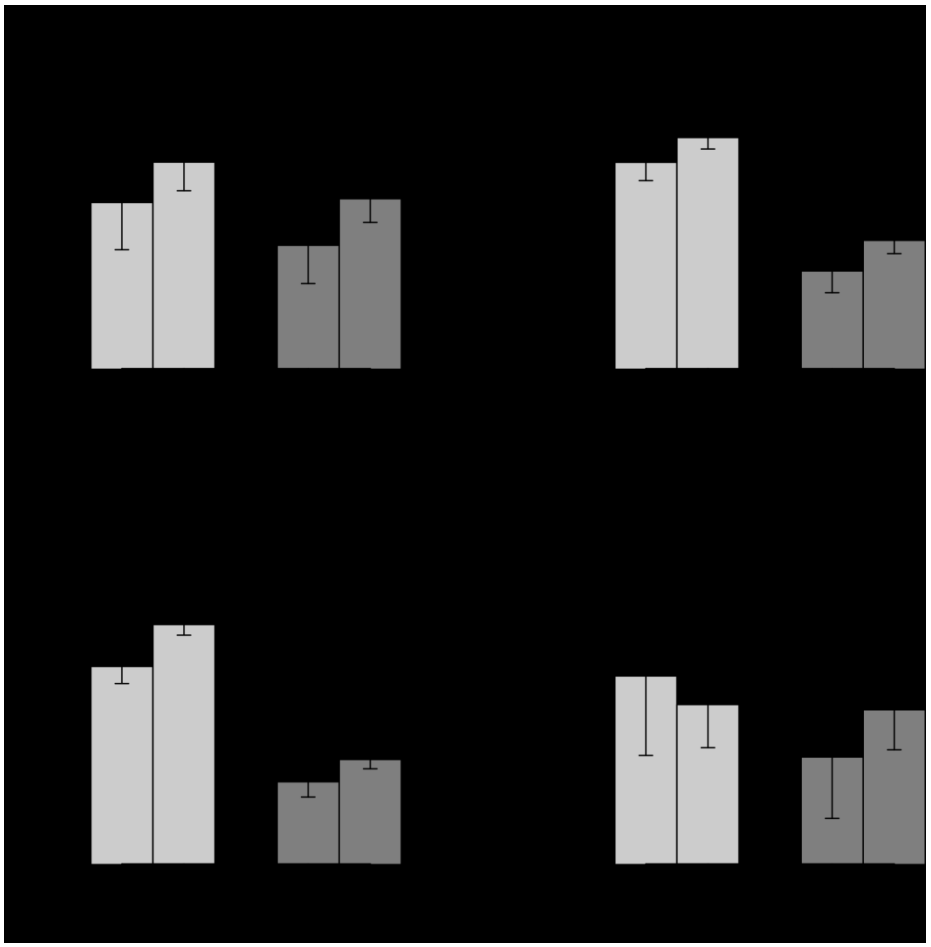
References

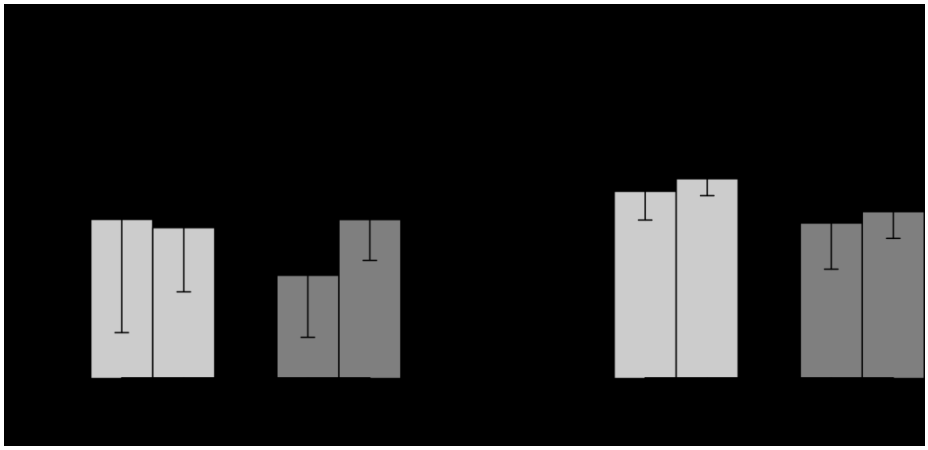
Hodges, J.S., 2013. Richly Parameterized Linear Models: Additive, Time Series, and Spatial Models Using Random Effects. CRC Press.

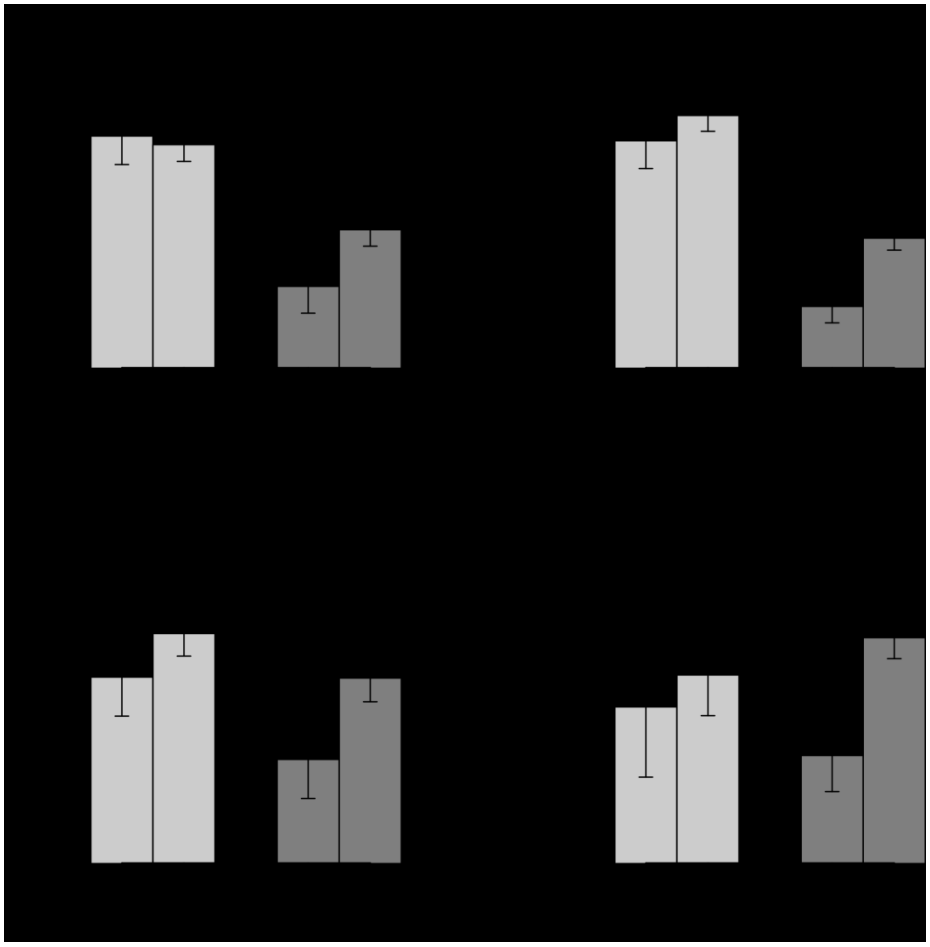
Appendix 6: Evolution of behaviour variability.

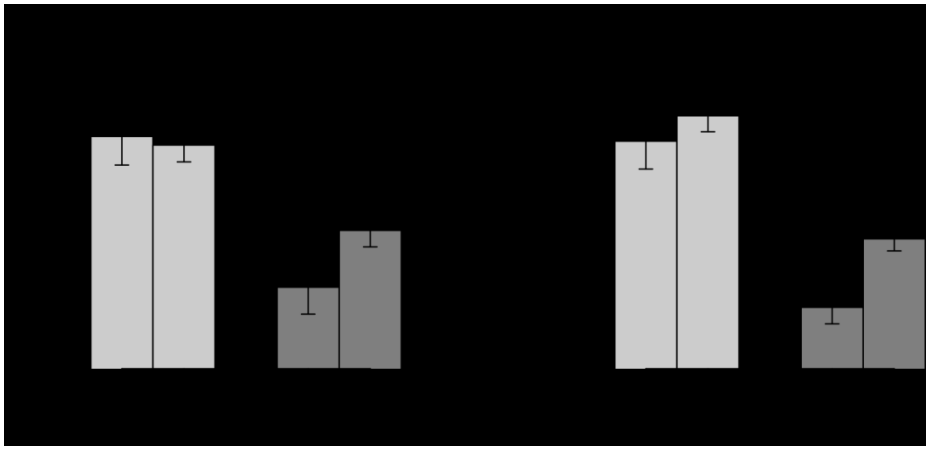
Below are the estimates given by the linear mixed models applied to each feature MAD and their respective standard errors for each of generations 0 and 50. Numbers in the x-axis represent the environments, defined by NaCl concentration. Asterisks highlight the statistical significant responses under a significance level of 0.05.

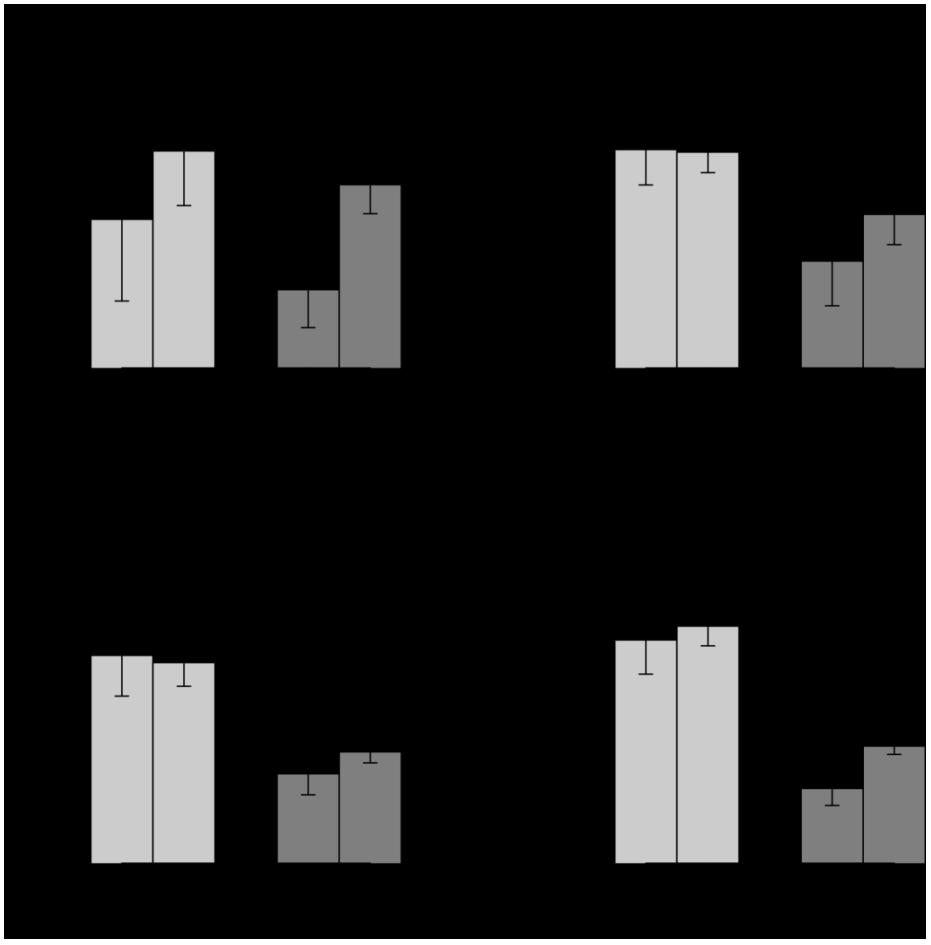
Appendix 6.1: Evolution of each behavioural feature MAD in the populations.

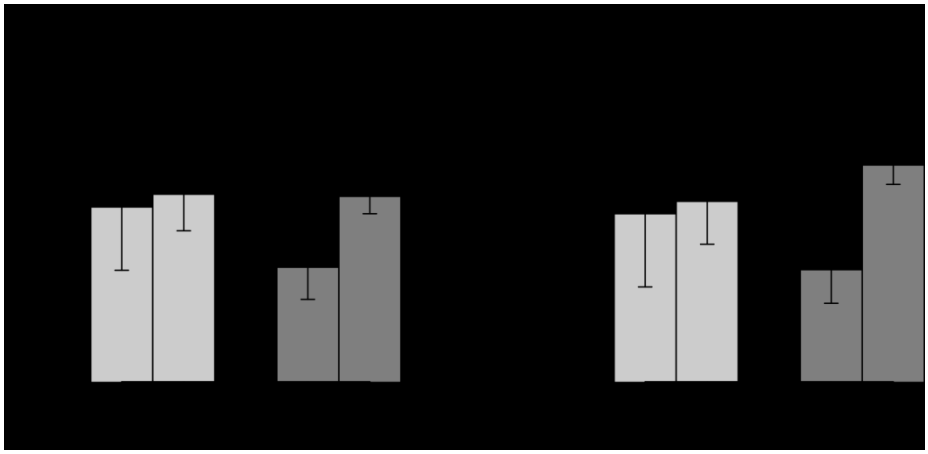


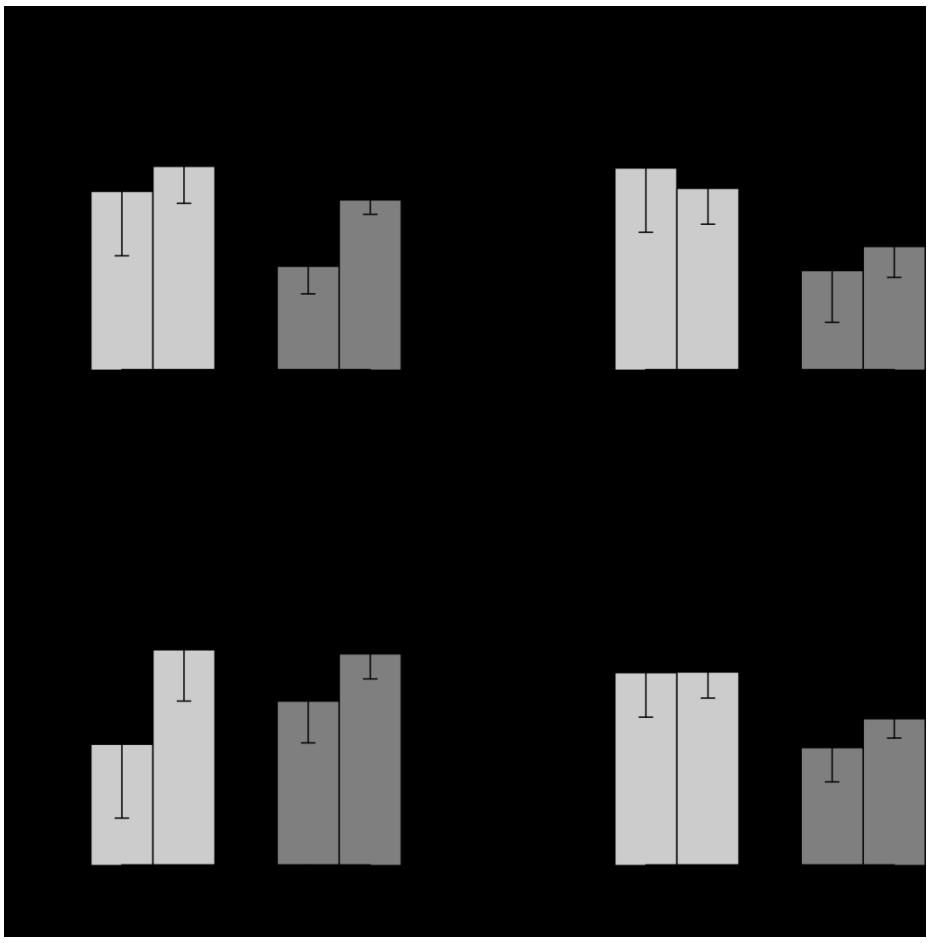


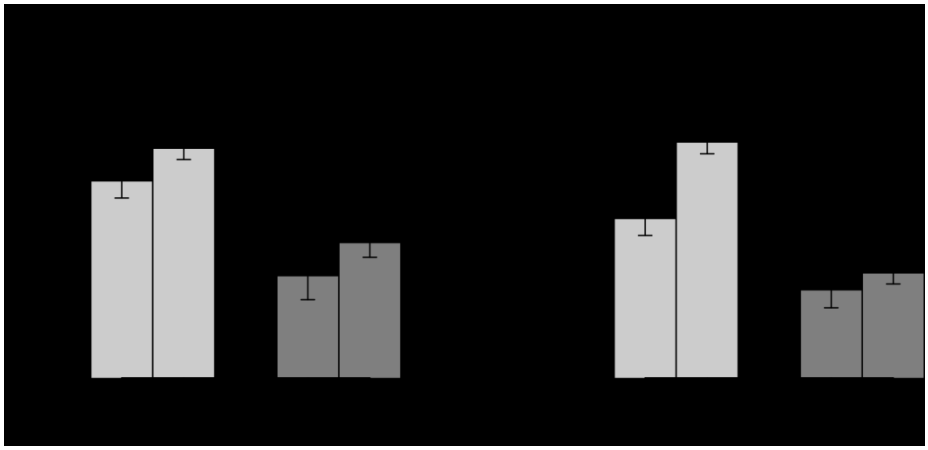


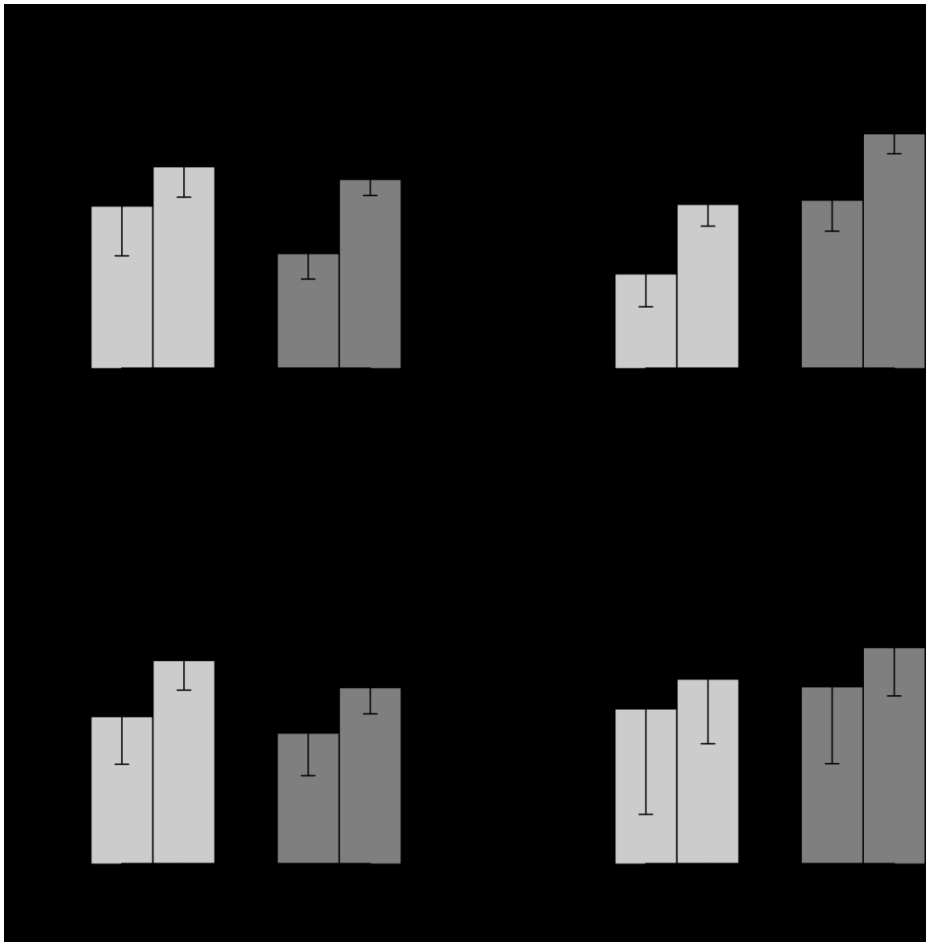


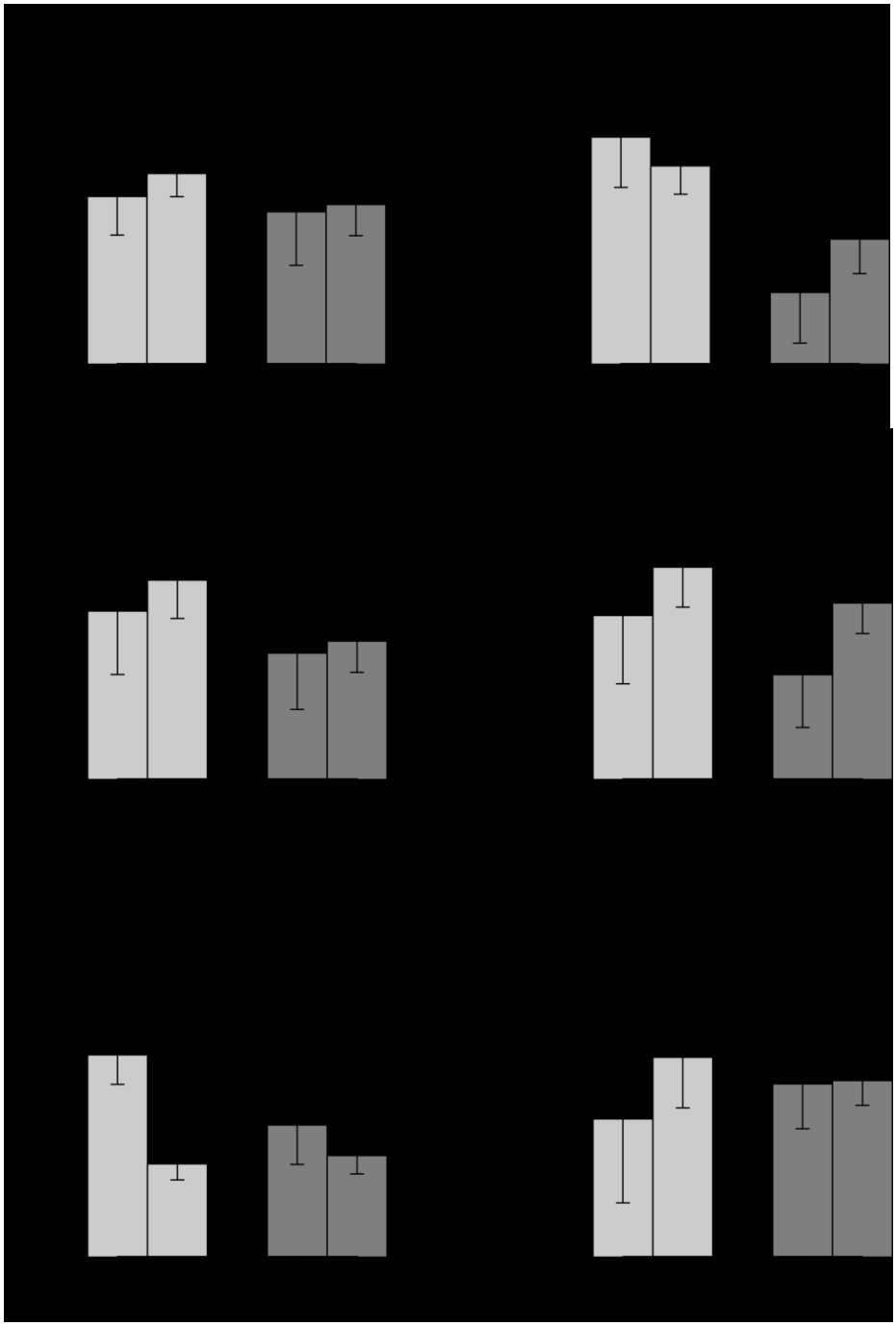




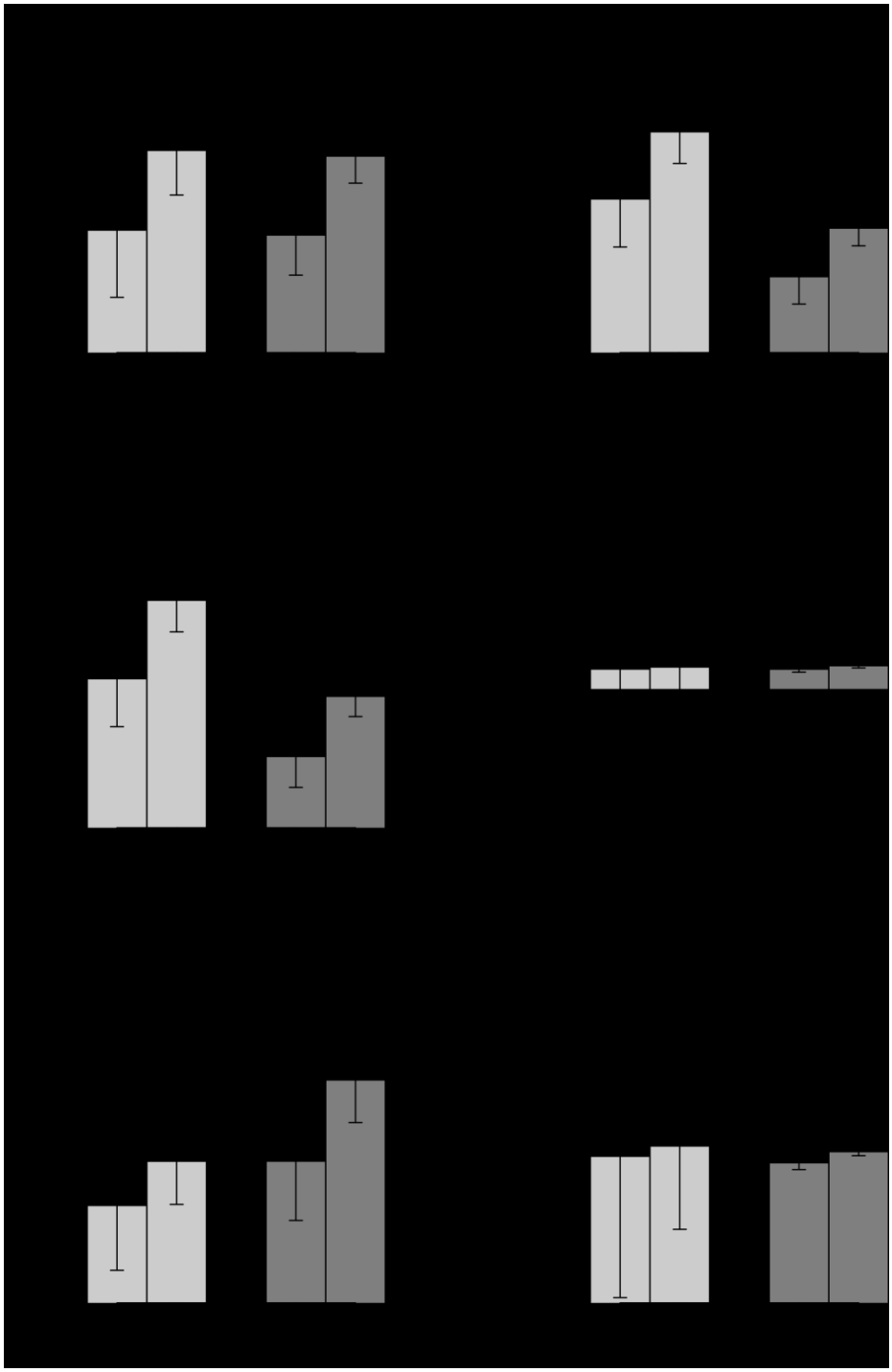


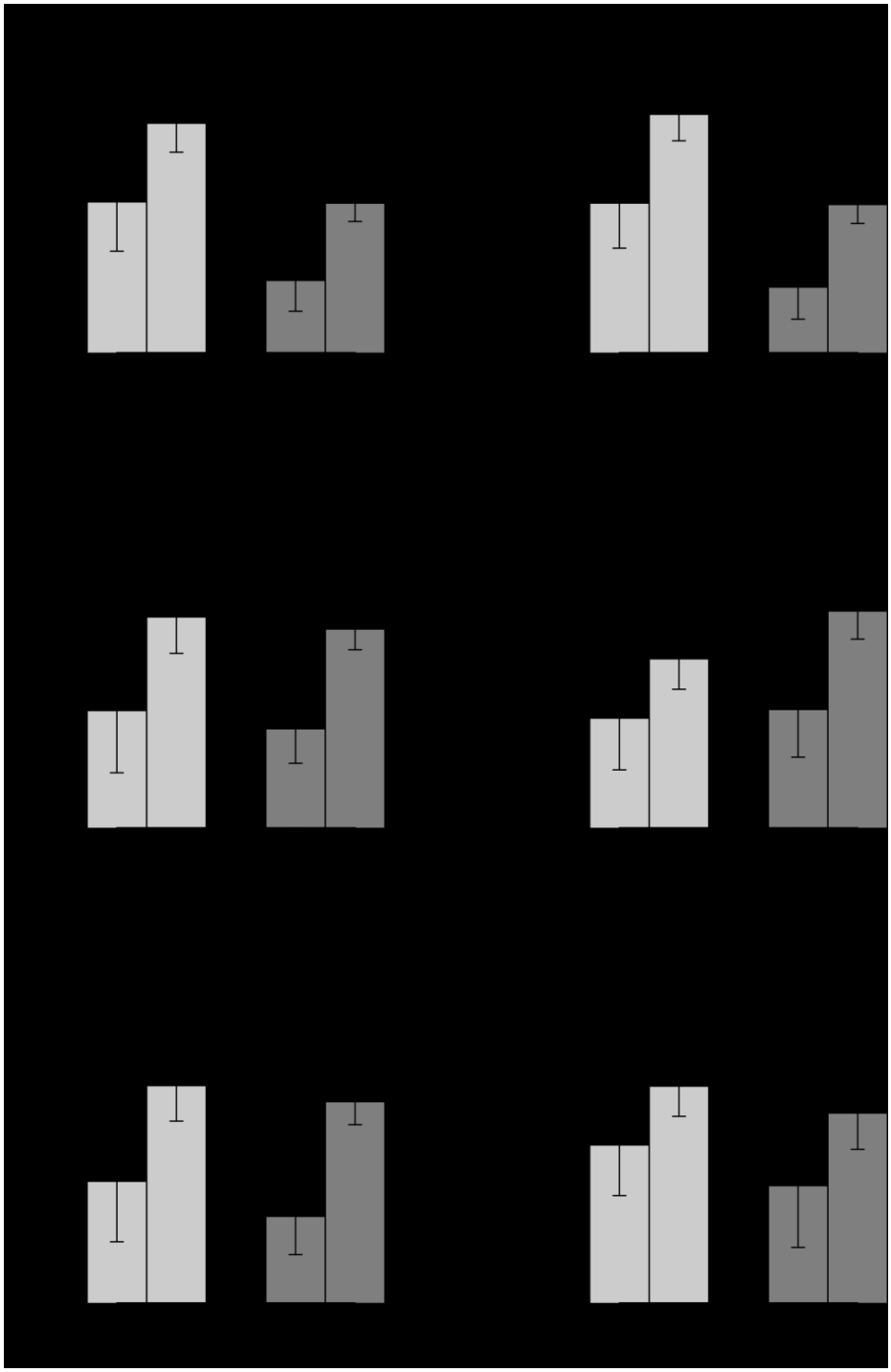


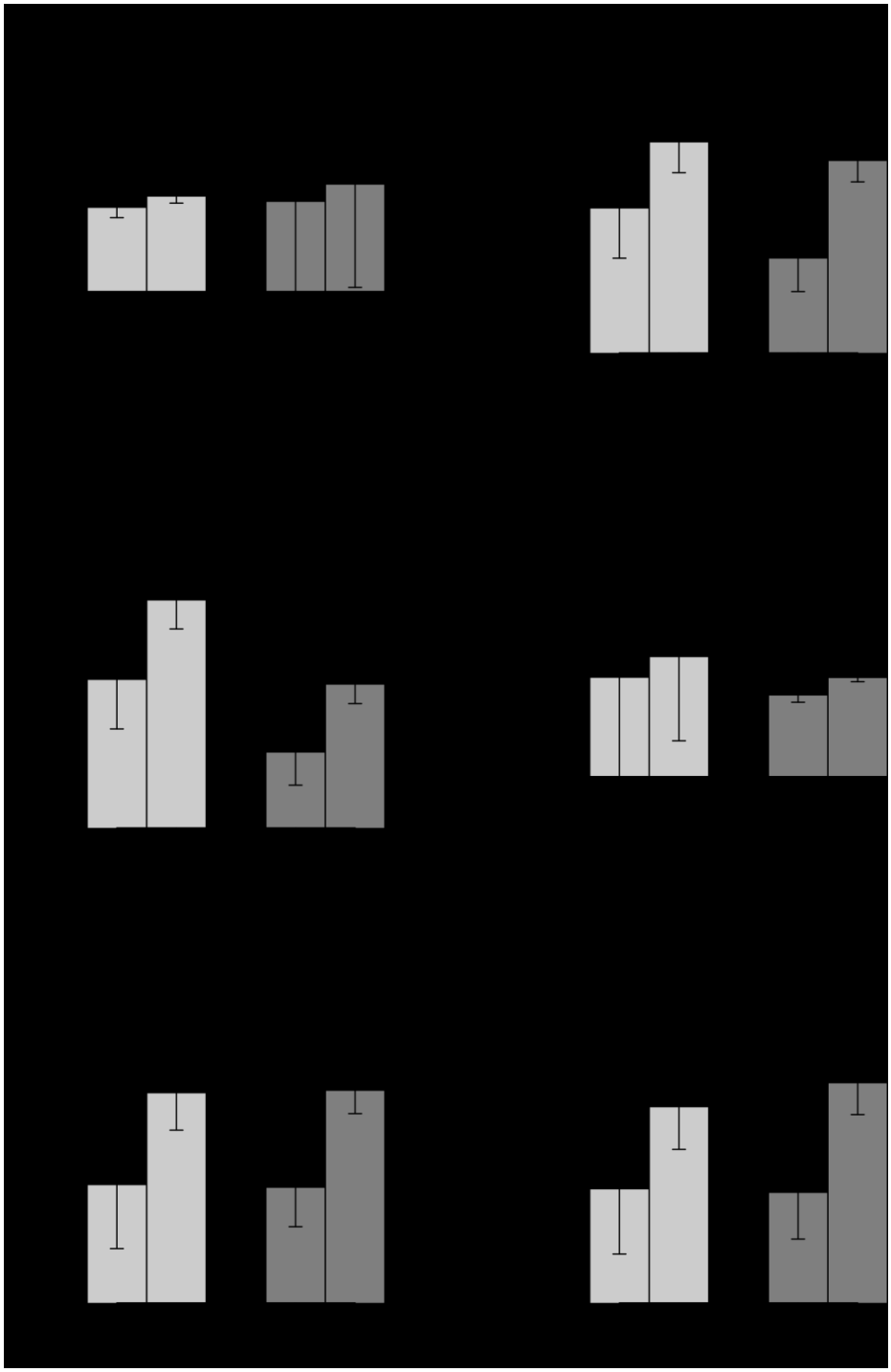


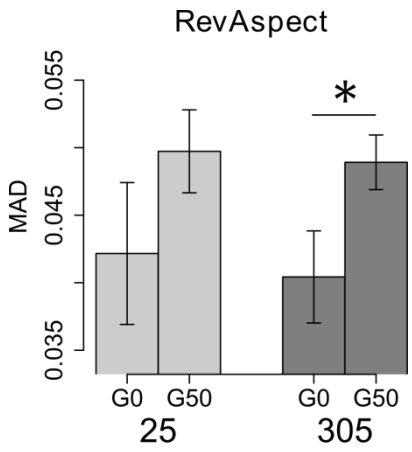


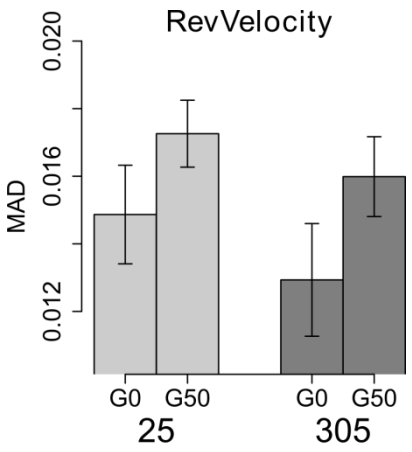
Appendix 6.2: Evolution of behavioural feature MAD in the inbred lines.

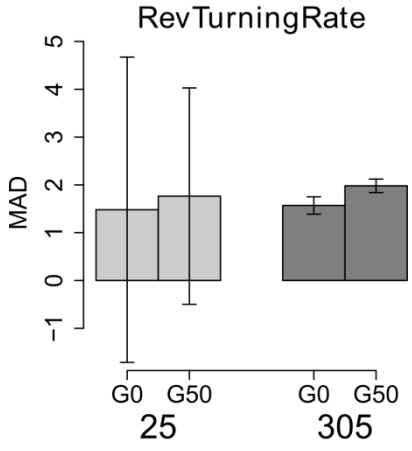


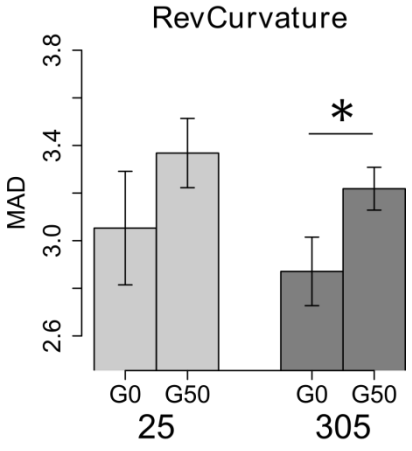


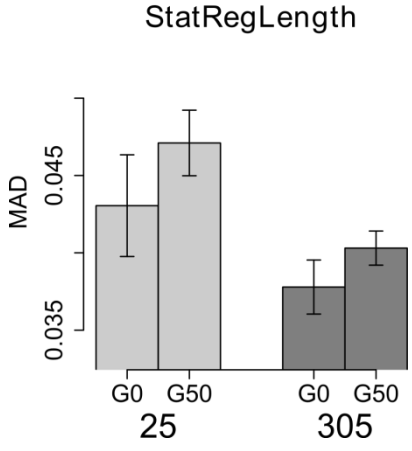


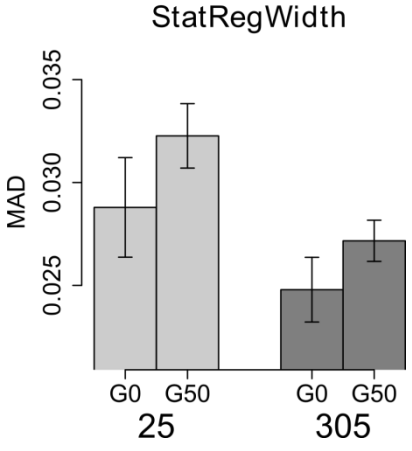

RevAspect
MAD
0.035
0.045
0.055
*
G0
G50
G0
G50
25
305

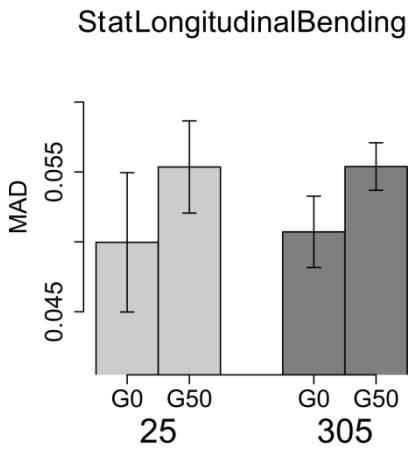

RevVelocity
MAD
0.012
0.016
0.020
G0
G50
G0
G50
25
305

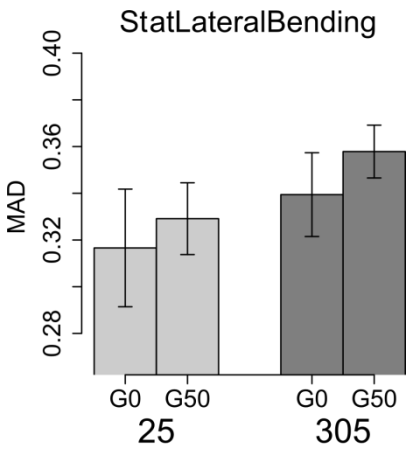

RevTurningRate
MAD
−1
0
1
2
3
4
5
G0
G50
G0
G50
25
305

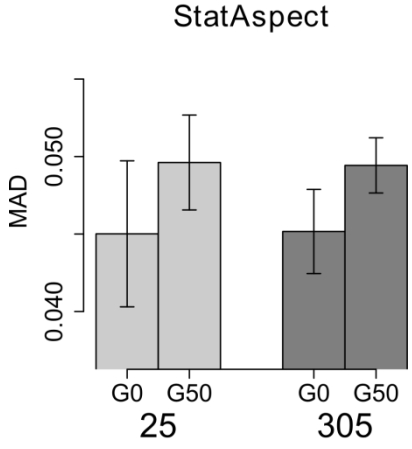

RevCurvature
MAD
2.6
3.0
3.4
3.8
*
G0
G50
G0
G50
25
305

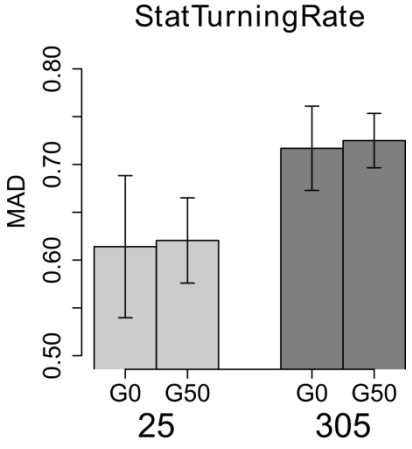

StatRegLength
MAD
0.035
0.045
G0
G50
G0
G50
25
305

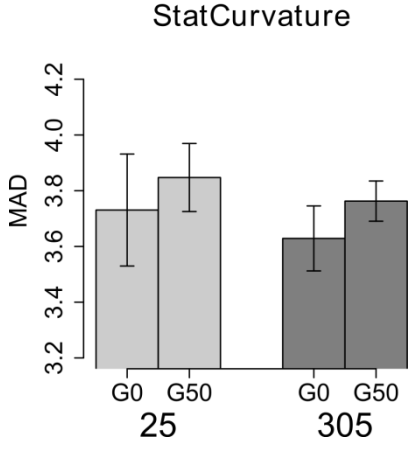

StatRegWidth
MAD
0.025
0.030
0.035
G0
G50
G0
G50
25
305

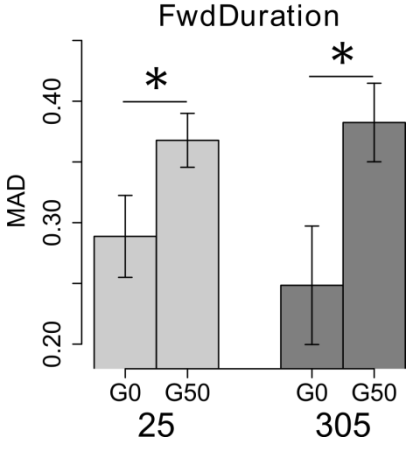

StatLongitudinalBending
MAD
0.045
0.055
G0
G50
G0
G50
25
305

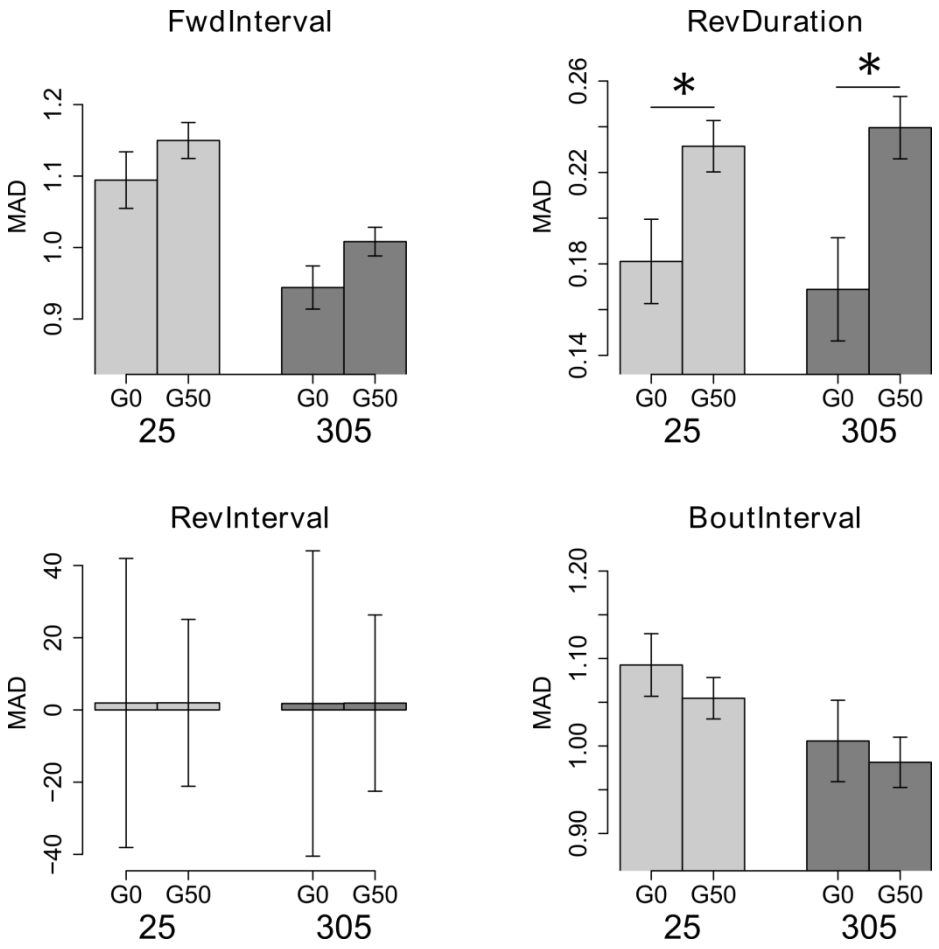

StatLateralBending
MAD
0.28
0.32
0.36
0.40
G0
G50
G0
G50
25
305


StatAspect
MAD
0.040
0.050
G0
G50
G0
G50
25
305


StatTurningRate
MAD
0.50
0.60
0.70
0.80
G0
G50
G0
G50
25
305


StatCurvature
MAD
3.2
3.4
3.6
3.8
4.0
4.2
G0
G50
G0
G50
25
305

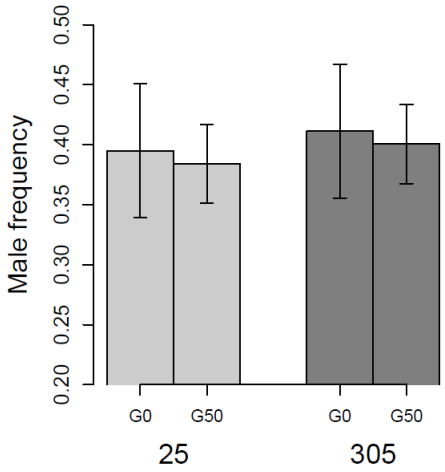

FwdDuration
MAD
0.20
0.30
0.40
*
*
G0
G50
G0
G50
25
305



Some of the least-squares estimates here shown are associated with disproportionate standard errors, as also been observed for behaviour centrality in the inbred lines (see Appendix 5.2).

Appendix 7: Male frequency in tracking plates of the populations.

The plot below shows estimates obtained by least-squares means under a linear mixed model with generation and environment as fixed factors and population as a random factor (NaCl 25 mM: t = 0.165; d.f.: 2.024; P = 0.884. NaCl 305 mM: t = 0.164; d.f.: 2.035; P = 0.885).



Appendix 8: Heritabilities estimated in the ancestral population.

P-values lower than 0.01 indicate that no bootstrap sample had a heritability higher than the observed in the original dataset. H^2_{25} represents heritability in NaCl 25 mM and H^2_{305} heritability in NaCl 305 mM.

Appendix 8.1: Heritabilities of behaviour centrality (median).

	H^2_{25}	Std. error	*P*
Aspect	0.0255	5.48×10^{-5}	< 0.01
RegressedLength	0.0762	3.19×10^{-5}	< 0.01
RegressedWidth	0.0366	3.88×10^{-5}	< 0.01
Velocity	0.0348	3.46×10^{-5}	< 0.01
TurningRate	0.0296	3.94×10^{-5}	< 0.01
Curvature	0.0506	3.70×10^{-5}	< 0.01
FwdRegLength	0.0739	3.23×10^{-5}	< 0.01
FwdRegWidth	0.0311	4.25×10^{-5}	< 0.01
FwdLongitudinalBending	0.0188	3.89×10^{-5}	< 0.01
FwdLateralBending	0.0279	4.77×10^{-5}	< 0.01
FwdAspect	0.0247	3.28×10^{-5}	< 0.01
FwdVelocity	0.0410	3.97×10^{-5}	< 0.01
FwdTurningRate	0.0336	3.55×10^{-5}	< 0.01
FwdCurvature	0.0418	4.37×10^{-5}	< 0.01
RevRegLength	0.0665	4.53×10^{-5}	< 0.01
RevRegWidth	0.0256	3.80×10^{-5}	< 0.01
RevLongitudinalBending	0.0150	4.71×10^{-5}	< 0.01
RevLateralBending	0.0212	3.66×10^{-5}	< 0.01
RevAspect	0.0191	4.13×10^{-5}	< 0.01
RevVelocity	0.0361	5.56×10^{-5}	< 0.01
RevTurningRate	0.0211	4.28×10^{-5}	< 0.01
RevCurvature	0.0360	4.56×10^{-5}	< 0.01
StatRegLength	0.0720	3.61×10^{-5}	< 0.01
StatRegWidth	0.0345	3.69×10^{-5}	< 0.01
StatLongitudinalBending	0.0209	3.66×10^{-5}	< 0.01
StatLateralBending	0.0312	3.23×10^{-5}	< 0.01
StatAspect	0.0268	3.87×10^{-5}	< 0.01
StatTurningRate	0.0330	3.08×10^{-5}	< 0.01
StatCurvature	0.0472	4.37×10^{-5}	< 0.01

FwdDuration	0.0611	3.99×10^{-5}	< 0.01
FwdInterval	0.0139	3.76×10^{-5}	< 0.01
RevDuration	0.0255	5.48×10^{-5}	< 0.01
RevInterval	0.00354	6.12×10^{-5}	< 0.01
BoutInterval	0.0303	4.20×10^{-5}	< 0.01
FwdFrequency	0.0254	3.81×10^{-5}	< 0.01
FwdFraction	0.0437	3.66×10^{-5}	< 0.01
FwdDistance	0.0440	4.05×10^{-5}	< 0.01
RevFrequency	0.0186	3.74×10^{-5}	< 0.01
RevFraction	0.0241	3.50×10^{-5}	< 0.01
RevDistance	0.0224	3.21×10^{-5}	< 0.01
LocomotionFraction	0.0476	3.32×10^{-5}	< 0.01
TotalDistanceTravelled	0.0484	4.46×10^{-5}	< 0.01
NetDistanceTravelled	0.0243	4.18×10^{-5}	< 0.01
ExplorationRate	0.0325	3.45×10^{-5}	< 0.01
ExplorationDensity	0.0280	3.19×10^{-5}	< 0.01
DistanceNearestAvgNeighbour	0.0176	3.54×10^{-5}	< 0.01
DistanceNearestAvgNeighbourStd	0.0433	4.03×10^{-5}	< 0.01

	H^2_{305}	Std. Error	*P*
Aspect	0.0578	4.67×10^{-5}	< 0.01
RegressedLength	0.0659	4.35×10^{-5}	< 0.01
RegressedWidth	0.0415	2.58×10^{-5}	< 0.01
Velocity	0.0337	3.31×10^{-5}	< 0.01
TurningRate	0.0300	3.49×10^{-5}	< 0.01
Curvature	0.0450	3.30×10^{-5}	< 0.01
FwdRegLength	0.0580	3.26×10^{-5}	< 0.01
FwdRegWidth	0.0333	3.25×10^{-5}	< 0.01
FwdLongitudinalBending	0.0211	3.15×10^{-5}	< 0.01
FwdLateralBending	0.0231	3.30×10^{-5}	< 0.01
FwdAspect	0.0313	3.56×10^{-5}	< 0.01
FwdVelocity	0.0579	3.14×10^{-5}	< 0.01
FwdTurningRate	0.0469	2.96×10^{-5}	< 0.01
FwdCurvature	0.0354	4.63×10^{-5}	< 0.01
RevRegLength	0.0481	4.30×10^{-5}	< 0.01
RevRegWidth	0.0222	4.30×10^{-5}	< 0.01
RevLongitudinalBending	0.0135	4.01×10^{-5}	< 0.01
RevLateralBending	0.0160	4.72×10^{-5}	< 0.01
RevAspect	0.0192	4.16×10^{-5}	< 0.01
RevVelocity	0.0444	4.18×10^{-5}	< 0.01
RevTurningRate	0.0240	5.02×10^{-5}	< 0.01
RevCurvature	0.0249	3.85×10^{-5}	< 0.01
StatRegLength	0.0625	3.44×10^{-5}	< 0.01
StatRegWidth	0.0387	2.73×10^{-5}	< 0.01
StatLongitudinalBending	0.0222	3.84×10^{-5}	< 0.01
StatLateralBending	0.0273	3.14×10^{-5}	< 0.01

StatAspect	0.0350	3.65×10^{-5}	< 0.01
StatTurningRate	0.0486	3.00×10^{-5}	< 0.01
StatCurvature	0.0434	3.62×10^{-5}	< 0.01
FwdDuration	0.0826	3.71×10^{-5}	< 0.01
FwdInterval	0.00907	3.86×10^{-5}	< 0.01
RevDuration	0.0578	4.67×10^{-5}	< 0.01
RevInterval	0.0118	5.98×10^{-5}	< 0.01
BoutInterval	0.0162	4.23×10^{-5}	< 0.01
FwdFrequency	0.0488	3.56×10^{-5}	< 0.01
FwdFraction	0.0479	3.15×10^{-5}	< 0.01
FwdDistance	0.0440	3.59×10^{-5}	< 0.01
RevFrequency	0.0294	3.15×10^{-5}	< 0.01
RevFraction	0.0301	4.00×10^{-5}	< 0.01
RevDistance	0.0220	3.23×10^{-5}	< 0.01
LocomotionFraction	0.0576	2.12×10^{-5}	< 0.01
TotalDistanceTravelled	0.0537	4.01×10^{-5}	< 0.01
NetDistanceTravelled	0.0221	3.87×10^{-5}	< 0.01
ExplorationRate	0.0263	3.00×10^{-5}	< 0.01
ExplorationDensity	0.0478	3.09×10^{-5}	< 0.01
DistanceNearestAvgNeighbour	0.0200	2.92×10^{-5}	< 0.01
DistanceNearestAvgNeighbourStd	0.0387	3.67×10^{-5}	< 0.01

Appendix 8.2: Heritabilities of behaviour variability (MAD).

	H^2_{25}	Std. error	*P*
AspectMAD	0.0149	3.69×10^{-5}	< 0.01
RegressedLengthMAD	0.0129	3.93×10^{-5}	< 0.01
RegressedWidthMAD	0.0169	4.17×10^{-5}	< 0.01
VelocityMAD	0.0357	3.31×10^{-5}	< 0.01
TurningRateMAD	0.0256	4.70×10^{-5}	< 0.01
CurvatureMAD	0.0143	3.60×10^{-5}	< 0.01
FwdRegLengthMAD	0.0199	3.47×10^{-5}	< 0.01
FwdRegWidthMAD	0.0238	3.33×10^{-5}	< 0.01
FwdLongitudinalBendingMAD	0.0150	3.45×10^{-5}	< 0.01
FwdLateralBendingMAD	0.0141	4.15×10^{-5}	< 0.01
FwdAspectMAD	0.0200	3.61×10^{-5}	< 0.01
FwdVelocityMAD	0.0221	2.62×10^{-5}	< 0.01
FwdTurningRateMAD	0.0203	4.37×10^{-5}	< 0.01
FwdCurvatureMAD	0.0254	3.54×10^{-5}	< 0.01
RevRegLengthMAD	0.0132	4.13×10^{-5}	< 0.01
RevRegWidthMAD	0.0143	3.21×10^{-5}	< 0.01

RevLongitudinalBendingMAD	0.0109	2.21×10^{-5}	< 0.01
RevLateralBendingMAD	0.00827	5.71×10^{-5}	< 0.01
RevAspectMAD	0.0137	4.75×10^{-5}	< 0.01
RevVelocityMAD	0.0122	4.41×10^{-5}	< 0.01
RevTurningRateMAD	0.0159	4.45×10^{-5}	< 0.01
RevCurvatureMAD	0.0147	4.60×10^{-5}	< 0.01
StatRegLengthMAD	0.00742	3.69×10^{-5}	< 0.01
StatRegWidthMAD	0.00710	4.06×10^{-5}	< 0.01
StatLongitudinalBendingMAD	0.0124	3.71×10^{-5}	< 0.01
StatLateralBendingMAD	0.0119	3.76×10^{-5}	< 0.01
StatAspectMAD	0.0119	4.01×10^{-5}	< 0.01
StatTurningRateMAD	0.0296	3.30×10^{-5}	< 0.01
StatCurvatureMAD	0.00863	3.27×10^{-5}	< 0.01
FwdDurationMAD	0.0225	4.60×10^{-5}	< 0.01
FwdIntervalMAD	0.00395	4.77×10^{-5}	< 0.01
RevDurationMAD	0.00919	3.12×10^{-5}	< 0.01
RevIntervalMAD	0.00102	5.28×10^{-5}	< 0.01
BoutIntervalMAD	0.00860	3.64×10^{-5}	< 0.01

	H^2_{305}	Std. error	P
AspectMAD	0.0162	3.81×10^{-5}	< 0.01
RegressedLengthMAD	0.00810	2.95×10^{-5}	< 0.01
RegressedWidthMAD	0.0151	2.38×10^{-5}	< 0.01
VelocityMAD	0.0320	3.15×10^{-5}	< 0.01
TurningRateMAD	0.0263	3.15×10^{-5}	< 0.01
CurvatureMAD	0.0106	3.08×10^{-5}	< 0.01
FwdRegLengthMAD	0.0178	3.68×10^{-5}	< 0.01
FwdRegWidthMAD	0.0262	4.66×10^{-5}	< 0.01
FwdLongitudinalBendingMAD	0.0142	3.24×10^{-5}	< 0.01
FwdLateralBendingMAD	0.0101	4.01×10^{-5}	< 0.01
FwdAspectMAD	0.0223	3.77×10^{-5}	< 0.01
FwdVelocityMAD	0.0115	2.35×10^{-5}	< 0.01
FwdTurningRateMAD	0.0176	4.10×10^{-5}	< 0.01
FwdCurvatureMAD	0.0270	2.78×10^{-5}	< 0.01
RevRegLengthMAD	0.00858	3.82×10^{-5}	< 0.01
RevRegWidthMAD	0.0141	3.44×10^{-5}	< 0.01
RevLongitudinalBendingMAD	0.00901	3.66×10^{-5}	< 0.01
RevLateralBendingMAD	0.00672	3.45×10^{-5}	< 0.01
RevAspectMAD	0.0129	4.62×10^{-5}	< 0.01
RevVelocityMAD	0.0104	5.61×10^{-5}	< 0.01
RevTurningRateMAD	0.0114	3.63×10^{-5}	< 0.01
RevCurvatureMAD	0.0127	4.78×10^{-5}	< 0.01
StatRegLengthMAD	0.00554	3.07×10^{-5}	< 0.01
StatRegWidthMAD	0.00664	1.97×10^{-5}	< 0.01
StatLongitudinalBendingMAD	0.0127	3.87×10^{-5}	< 0.01
StatLateralBendingMAD	0.00889	3.41×10^{-5}	< 0.01

StatAspectMAD	0.0129	3.29×10^{-5}	< 0.01
StatTurningRateMAD	0.0422	3.32×10^{-5}	< 0.01
StatCurvatureMAD	0.00697	4.04×10^{-5}	< 0.01
FwdDurationMAD	0.0287	4.03×10^{-5}	< 0.01
FwdIntervalMAD	0.00412	3.68×10^{-5}	< 0.01
RevDurationMAD	0.0124	3.32×10^{-5}	< 0.01
RevIntervalMAD	0.00102	6.44×10^{-5}	< 0.01
BoutIntervalMAD	0.00482	3.66×10^{-5}	< 0.01

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