Inversion frequencies and phenotypic effects are modulated by the environment

Emma Berdan[1], Hanna Rosenquist[2], Keith Larson[3], Maren Wellenreuther[4,5,*]

[1]Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

[2] MEMEG, Ecology Building, Lund University, Lund, Sweden

[3]Department of Ecology and Environmental Sciences, Climate Impacts Research Centre, Umeå University, Umeå, Sweden

[4]The New Zealand Institute for Plant & Food Research Ltd, Auckland, New Zealand

[5]School of Biological Sciences, the University of Auckland, New Zealand

*Corresponding author: Maren Wellenreuther, The New Zealand Institute for Plant & Food Research Limited, Box 5114, Port Nelson, Nelson 7043, New Zealand.

tel.: +64 3 989 7658; fax: +64 3 546 7049; e-mail: maren.wellenreuther@plantandfood.co.nz

Keywords: *Coelopa frigida*, inversion polymorphism, adaptation, GxE interactions, frequency effects

Running title: karyotype x environment effects

Word count: 4,709 (total), 224 (Abstract), 867 (Introduction), 1560 (Materials and Methods), 710 (Results), 1259 (Discussion).

Cited References: 72

Figures and Tables: 4 Figures, 3 Tables

Abstract

Understanding how environmental variation drives phenotypic diversification within species is a major objective in evolutionary biology. The seaweed fly *Coelopa frigida* provides an excellent model for the study of genetically driven phenotypes because it carries an α/β inversion polymorphism that affects body size. *Coelopa frigida* inhabits highly variable beds of decomposing seaweed on the coast in Scandinavia thus providing a suitable test ground to investigate the genetic effects of substrate on both the frequency of the inversion (directional selection) and on the phenotype (genotype x environment effects). Here we use a reciprocal transplant experiment to test the effect of the α/β inversion on body size traits and development time across four suitable natural breeding substrates from the clinal distribution. We show that while development time is unaffected by GxE effects, both the frequency of the inversion and the relative phenotypic effects of the inversion on body size differ between population x substrate combinations. This indicates that the environment modulates the fitness as well as the phenotypic effects of the inversion karyotypes. It further suggests that the inversion may have accumulated qualitatively different mutations in different populations that interact with the environment. Together our results are consistent with the idea that the inversion in *C. frigida* likely evolves via a combination of local mutation, GxE effects, and differential fitness of inversion karyotypes in heterogeneous environments.

Introduction

One of the central goals of evolutionary biology is to understand how organisms cope with environmental heterogeneity (Savolainen et al. 2013). Phenotypic plasticity can solve this problem but the phenotypic response to variation in an environmental variable (i.e. phenotypic plasticity) may vary among species as well as among genotypes of the same species. This is commonly referred to as a Genotype-by-Environment interaction (GxE, Falconer 1990; Lynch and Walsh 1998; Via and Lande 1985). Abundant experimental data and theoretical work now provide strong support for the idea that the relative performance of different genotypes across environmental gradients may be involved in the maintenance of phenotypic plasticity and genetic variation as well as the evolution of fitness related phenotypes (Carreira et al. 2006; Ungerer et al. 2003).

The study of environmental heterogeneity has been championed by work on geographic patterns along clines, with classic examples coming from genetic clines in flies almost a century ago (Dobzhansky 1948; Sturtevant 1921). Other examples include the genetic underpinnings of traits involved in adaptation to high altitude (Storz 2002) and pigmentation (Hoekstra and Coyne 2007). Clines hold a large attraction as natural Darwinian laboratories because they allow researchers to quantify the gradual effects of changing environments on genotype, phenotype, and the interaction between them (Endler 1977). These studies have shown that while many environmental tolerance traits that vary

68 clinally are quantitative and polygenic (Wellenreuther and Hansson 2016), they
69 can also be located within chromosomal segments that segregate as a single
70 locus (Tigano and Friesen 2016). If environmentally adaptive variants are
71 chromosomally linked, either because they are found on chromosomal inversions
72 or are in areas of low recombination, their selective coefficients act in an additive
73 manner and they may be less likely to be lost via genetic drift and more likely to
74 contribute to rapid adaptation (Kirkpatrick and Barton 2006). In the extreme case,
75 complex adaptations may be accomplished through the fixation of linked sets of
76 adaptive variants (Yeaman 2013), often called supergenes, a case in which
77 polygenic adaptation may be more accurately modeled with single-locus models
78 (Schwander et al. 2014). Good examples for such single-locus supergene
79 adaptations come from *Drosophila melanogaster* where inversion polymorphisms
80 produce latitudinally varying phenotypes for traits including heat and cold
81 tolerance and body size (Reinhardt et al. 2014; Schrider et al. 2016).

82 Chromosomal inversion polymorphisms are being identified at a fast rate from an
83 increasing number of populations of organisms, e.g. insects, plants, bacteria and
84 humans, and are commonly linked to trait and fitness variation (Hoffmann and
85 Rieseberg 2008; Kirkpatrick and Barton 2006). Inversions are particularly
86 common in flies, and an intriguing mystery is why there are huge differences in
87 levels of polymorphism and rates of fixation for inversions between closely
88 related species and even between chromosomes in *Drosophila* spp. (Kirkpatrick
89 2010). The seaweed fly *Coelopa frigida* (family Coelopidae) and its sister species
90 *C. nebularum* have a large chromosomal α/β inversion polymorphism on

91 chromosome I encompassing about 10% of the genome and containing ~100
92 genes (Aziz 1975). The inversion polymorphism is known to affect a wide range
93 of life history and reproductive traits, such as development time (Day and
94 Buckley 1980), body size (Butlin et al. 1982a), longevity and female fecundity
95 (Butlin and Day 1984a; Butlin et al. 1982c), female resistance to male mating
96 attempts (Gilburn and Day 1994; Gilburn et al. 1992) and also male's ability to
97 overcome female resistance (Butlin et al. 1982a).

98 The most striking of these phenotypic traits is a three-fold size difference in
99 males: male homokaryotypes for the inversion type α are much larger (~9mm)
100 than β- homokaryotypes (~3mm), whereas the heterokaryotype αβ is
101 intermediate in size (~6mm) (Figure 1a, Wellenreuther pers. obs., Butlin and Day
102 1984b). Female size is not significantly affected by karyotype (Butlin and Day
103 1984b). Maintenance of the polymorphism is secured by strong heterosis (Collins
104 1978), since αβ-heterokaryotypes have higher fitness than either homokaryotype
105 (Butlin and Day 1984a; Butlin and Day 1989). The heterokaryotype αβ has a
106 higher viability during development than either homokaryotype, both in
107 experiments (Butlin and Day 1984a; Leggett et al. 1996) and in the field, as
108 indicated by heterokaryotype excess (Butlin et al. 1982c; Day et al. 1983; Day et
109 al. 1982b; Gilburn and Day 1994). Heterokaryotype excess increases under
110 interspecific competition with the congener *C. pilipes* and under intense
111 intraspecific competition (Butlin and Day 1984a; Leggett et al. 1996). The effect
112 is disproportionally strong in males, especially decreasing the frequency of αα-
113 homokaryotypes (Butlin and Day 1984a), possibly indicating lower viability of

114 males of this karyotype. However, inversion frequencies also form a cline from
115 the North Sea to the Baltic Sea, with the α inversion becoming more rare towards
116 the Baltic Sea (Day et al. 1983).

117 Here we explore if the environment modulates inversion frequencies and
118 phenotypic effects in *C. frigida*. For this, we study *C. frigida* populations in
119 Scandinavia that show variation in karyotype frequencies (Day et al. 1983).
120 Specifically, we test the possible effects of the α/β inversion polymorphism on
121 body traits and development time across four suitable natural breeding
122 substrates using a fully balanced reciprocal transplant experiment. We also
123 elucidate the relative fitness of the inversion karyotypes on these varying
124 substrates by examining the frequencies of the three karyotypes in different
125 environments.

126 Materials and methods

127 Study species and sites

128 *Coelopa frigida* belongs to the group of acalyptrate flies which exclusively forage
129 on decomposing seaweed (Cullen et al. 1987) and is found all along the
130 seashores of Scandinavia (Mcalpine 1991). This species plays a vital role in
131 coastal environment health by accelerating the decomposition of algae, allowing
132 for faster release of nutrients (Cullen et al. 1987), and are an important food
133 source for predatory coastal invertebrates and seabirds (Fuller 2003; Summers
134 et al. 1990). *Coelopa frigida* has 5 chromosomes with inversions occuring on 3 of
135 them (Aziz 1975). The largest of these is the α/β which occurs on chromosome I.

136 The α/β inversion occurs at stable frequencies in all populations so far studied in
137 Northern Europe (Butlin et al. 1982a), although there may be seasonal (Butlin
138 1983) and clinal variation (Day et al. 1983).

139 Inversion frequencies of *C. frigida* form a cline from the North Sea to the Baltic
140 Sea, with the α inversion becoming more rare towards the Baltic Sea (Day et al.
141 1983). Such clines are classic signs of selection and this cline is associated with
142 a decrease in salinity and tidal range and associated changes in the composition
143 of the wrackbed (i.e. the species of algae present). As salinity decreases from
144 the North Sea to the Skagerrak and Kattegat regions there is a corresponding
145 decrease of marine species of brown seaweeds such as *Fucus spp*. and
146 *Laminaria spp.* moving into the Baltic Sea, *Zostera* eelgrass and red algae
147 gradually displace the brown seaweeds (Table 1). We selected four populations
148 (Kämpinge, Kåseberga, Mölle and Steninge) from the southern part of the cline
149 that differ in tidal range, salinity, and wrackbed composition (Figure 1b, Table 1).
150 We extracted climate data (sea surface salinity and air temperature) for each site
151 from MARSPEC (Sbrocco and Barber 2013) and WorldClim databases using the
152 R package raster (Hijmans and van Etten 2014) (Table 1).

153 **Reciprocal transplant experiment**

154 We conducted a fully balanced reciprocal transplant experiment involving all four
155 locations, where larvae from all locations were allowed to grow on its own
156 wrackbed substrate and the substrates of the remaining three locations. For each
157 location, fly larvae and wrackbed substrate were collected between February and

158 May 2014. The wrackbed substrate was placed in labelled plastic bags and then
159 frozen to ensure that no viable eggs or larvae remained. Fly larvae from were
160 reared on 150g of their own substrate in aerated plastic containers until a
161 puparium was formed, and then kept in individual Eppendorf tubes (to ensure
162 virginity) containing a small piece of cotton soaked in 5% sucrose solution. When
163 a fly eclosed, the Eppendorf was moved to the refrigerator to slow down aging.
164 *Coleopa frigida* is exceptionally cold tolerant and this treatment does not appear
165 to affect survival (E. Berdan, pers. obs.). Over a maximum of ten days, adult flies
166 were collected until a minimum of 25 couples could be set up per location. Eggs
167 were collected from 14-15 mating pairs per population and subsequently mixed
168 to populate three replicates of each population x substrate combination with 30
169 eggs each (4 populations x4 substrates x 3 replicates, each one with 30 eggs,
170 total eggs for the experiment: 1440). Replicate pots were kept at 25° C with a
171 12/12 hour light:dark cycle and each pot (190x110x70 mm) was aerated and
172 contained 150 g of seaweed. Pots were checked daily and all eclosing flies were
173 sexed and then stored at -20° C on the same day.

174 **Morphological measurements**

175 Morphological measurements were carried out on adult flies only. All flies were
176 weighed (Sartorius Quintix 124-1S microbalance) to the nearest 0.0001 grams.
177 The thorax width of each fly was measured with a digital calliper (Cocraft Digital
178 Vemier Caliper) at the widest place and to the nearest 0.01 mm. This
179 measurement was repeated twice and the average was used. Then, the left wing
180 and right middle leg were removed (if one side was damaged the other side was

181 used). The wing and leg were placed under a thin glass slide and placed under
182 the microscope and a picture was the taken using a Nikon Digital Sight DS-Fi2
183 mounted on a Nikon SM2800 microscope set to a magnification of two power.
184 The resulting image was labelled with an individual identifier. Measurements
185 were conducted blind to location and substrate type to avoid bias.

186 **Molecular determination of inversion types**

187 In *C. frigida* the two most common alcohol dehydrogenase (Adh) allozymes, Adh-
188 B and Adh-D are in complete linkage disequilibrium with the α and β forms of the
189 inversion (Day et al. 1982a). Gel electrophoresis was used to visualise the
190 alcohol-dehydrogenase allozymes associated with the two inversion types and
191 this allowed for straightforward genotyping of the inversion types. Starch gels
192 (12%) were prepared by combining 24.5 g potato starch (Sigma-Aldrich, S4251)
193 with 90 ml TEB-buffer and 90 ml dH_2O, heating the mixture, and then pouring it
194 onto an 18.5x10 cm glass plate with a plastic frame and left to set for 30 minutes,
195 after which it was put in a $4^{O}C$ refrigerator overnight.

196 Porcelain plates with 12 wells were kept on ice packs. On each well, a fly was
197 placed together with a small amount of carborundum powder (Fisher Chemical,
198 #C192-500) and one drop of cold dH_2O. The flies were then homogenized using
199 a glass rod. Once homogenized, filter paper wicks were placed in the wells to
200 soak up the homogenate. The wicks were then gently inserted into the starch gel.

201 Starch gels were put in the electrophoresis chamber, which was connected to a
202 power supply (Consort E863). The two chambers of the electrophoresis machine

203 were filled with TEB-buffer. Two cloth wicks were soaked in TEB-buffer and
204 placed so that they touched evenly along each of the exposed sides of the gel,
205 while still connected to the buffer. An ice pack was placed on top of the gel and
206 the electrophoresis (350 V, 50mA) took 1.5 hours.

207 After electrophoresis, the gel was cut horizontally with a metal thread and only
208 the bottom half was used for staining. The agar overlay used for staining
209 consisted of 20 ml of 2% agar, 10 ml HCl-Tris buffer (pH 8.6), 6 ml propan-2-ol,
210 1.5 ml 3-(4,5-dimethylthiazolyl-2)-2,5- diphenyltetrazolium bromide (MTT), 1 ml
211 Phenazine methosulphate (PMS), and 10 ml Nicotinamide-adenine dinucleotide
212 (NAD). The stain was mixed and poured onto the cut surface of the gel. The
213 covered box with the gel was then carefully put in a dark heating cabinet at 37° C
214 for 40 minutes, or until the banding pattern was clear and then photographed.

215 **Statistical analysis**

216 We analyzed weight and development time (in days) in R (R Development Core
217 Team 2015) using general linear models. Weight was highly correlated with all
218 other morphological characters (Supplemental table S1) so we only analyzed
219 weight data and development time in a statistical framework. Weight data was
220 normally distributed and so was analyzed assuming a Gaussian distribution.
221 Development time is essentially count data and right skewed, so we analyzed the
222 data assuming a Poisson distribution. For both data sets we considered
223 individual flies to be our unit of replication and used the lme4 package in R
224 (Bates et al. 2014) to construct a model with all factors (population, substrate,

225 sex, and karyotype) and all two and three way interactions. We also included pot
226 (replicate) nested within population*substrate as a random factor. We ran the
227 'Anova' function in the R package car (Fox and Weisberg 2011) on the full
228 models and then examined the F-values for each term. Starting with a model with
229 only an intercept we built up a series of nested models adding a single main
230 effect, two or three way interaction at a time by decreasing F-value (i.e. starting
231 with the term with the highest F-value). We then used the 'Anova' function with
232 the χ^2 distribution to compare the models in a nested fashion (i.e. each model is
233 only compared to the one above it) and used a χ^2 test to determine if they were
234 significantly different. We selected the model with the lowest AIC whose nested
235 models were not significantly better.

236 Visual inspection of our morphological data indicated that males were more
237 variable than females. We formally tested for differences in variability for each
238 morphological measurement in a statistical framework using Krishnamoorthy and
239 Lee's (2014) modified signed-likelihood ratio test (MS-LRT) implemented in the R
240 package cvequality (Marwick and Krishnamoorthy 2016). We used a Bonferroni
241 correction to account for multiple testing (Bland and Altman 1995).

242 We further examined the relative proportions of the three karyotypes (αα, αβ, and
243 ββ) in all population x substrate combinations. For these analyses we used pot
244 as our unit of replication. We tested for differences in two ways. First, we tested
245 for karyotype differences using a χ^2 test on a 3 karyotypes x 16 population-
246 substrate combination matrix. Second, we used a Dirichlet regression model in
247 the R package DirichletReg (Maier 2014). The Dirichlet regression model allows

us to analyze karyotypes (variables with a bounded interval that sum up to a constant - in our case frequencies of karyotypes in a population) exhibiting skewness and heteroscedasticity, without having to transform the data (Maier 2014). We used a nested approach starting with a simple model and then adding factors. Models were tested against the one above them in a nested fashion using the 'Anova' function in the R package car (Fox and Weisberg 2011) to determine the best model.

Results

Phenotypic data

We had different sample sizes for different treatment combinations (see figure legends for Figures 3 and 4) due to differential survival of the three karyotype categories. Three of the 96 combinations had zero flies (ex: αα Kämpinge females in Kämpinge) but we do not expect that this unduly influenced our results as we did not investigate any 4 way interactions in any of our statistical models and thus did not examine these three combinations directly. All morphological characteristics were highly and significantly correlated with each other (Supplementary Table S1).

We only examined weight in a statistical framework, as this morphological trait is a good proxy of the other morphological measurements (Pearson correlation coefficients with other traits varied from 0.78-0.89, Supplemental Table S1) while still being an easily tractable and understandable measurement. Weight was not strongly correlated with development time (Pearson's product-moment

correlation: 0.10) and therefore development time was examined separately. F-values from the full models indicated that effect sizes were much larger for weight than development time (Table S2). The best model for weight included all main effects, the two-way interactions: sex*karyotype, population*sex, population*karyotype, population*substrate, substrate*karyotype, and the three-way interaction: location*sex*karyotype. Comparing the best model with the next most simplified model gave a χ^2 value of 43.3 (P = 0.00073). The best model for development time included the main effects and the two-way interaction karyotype*sex. Comparing the best model with the next most simplified model gave a χ^2 value of 8.6 (P = 0.0135). The best models for both weight and development time included all main effects and a subset of interactions (Table 2). However, a GxE interaction (as evidenced by a population x substrate interaction) was only present for morphological characteristics, not for development time.

For all measured traits, the Krishnamoorthy and Lee's (2014) modified signed-likelihood ratio test (MS-LRT) showed that males had significantly more phenotypic variation than females (Table 3). This variation could be partially accounted for by karyotype. Previous work has shown that males are on average bigger than females and that within male size differences are primarily driven by the αβ inversion system: αα males are the biggest followed by αβ and ββ (Butlin et al. 1982c). This held true in our male data set (Figure 2) but the magnitude of these differences depended on both population and substrate type (Figure 2, Supplementary Figures 2-5). Although the relative sizes and size differences of

293 the karyotypes differed between combinations of location and substrate, the
294 pattern of both leg length and wing length, thorax width, and weight (Figure 2,
295 Supplementary Figures 2-5) in relation to karyotype and sex was clear and
296 consistent for males, but much less pronounced for females. Likewise, across
297 treatments (combination of population and substrate), αα-males had the largest
298 mean size, followed by αβ-males and ββ-males in all different population x
299 substrate combinations (Figure 2). For females, less than half of the
300 combinations of population and substrate followed the same pattern.

301 Development time did not follow the same pattern as observed for morphology
302 (Figure 3). While αα-males generally took longer to develop, this was not always
303 the case for every combination of population and substrate. For example, ββ flies
304 from Steninge, raised on Kämpinge substrate took longer to develop than any
305 other flies from Steninge. For females, the MS-LRT analysis demonstrated that
306 there was considerably less variation between karyotypes compared to males
307 and patterns varied substantially between populations and substrates.

308 **Inversion types**

309 The frequencies of αα, αβ, and ββ were significantly different across the
310 Population x Substrate combinations (χ^2 = 71.5, df=30, P < 0.0001). Generally
311 αβ was the most frequently observed karyotype, but ββ could be just as frequent
312 or more frequent depending on the substrate. In ¾ of the populations (all except
313 Steninge) ββ was the most frequent karyotype in at least one substrate and that
314 was usually the substrate from Mölle.

315 We used Dirichlet regression to see which parameters best predicted the
316 karyotype frequencies. With this model we were not able to examine F-values so
317 we compared all possible models with the 'Anova' function from the R package
318 car (Fox and Weisberg 2011). The best model was population + substrate +
319 population*substrate. Comparing this with the next most simplified model gave a
320 χ^2 value of 73.99 (P = 0.0002).

321 Discussion

322 Our results demonstrate that the environment modulates both the frequencies of
323 the karyotypes as well as their phenotypic effects in *C. frigida*. The presence of a
324 significant GxE term for both of these indicates that there is population specific
325 variation in larval fitness of the different inversion types as well as in the
326 phenotypic effects of the inversion. The same pool of eggs was used to seed
327 every substrate treatment per population so the neutral expectation (without
328 sampling bias) would be similar karyotype frequencies across substrates.
329 Instead, we found strong differences in karyotype frequencies between
330 substrates with some trends being consistent across multiple populations (ex:
331 higher frequency of ββ in Mölle substrate, Figure 4). While it is possible that
332 these results are due simply to sampling bias, it is unlikely that sampling bias
333 would produce consistent trends. Furthermore, this result confirms previous
334 research showing that the substrate composition has a strong selective effect on
335 the inversion (Butlin et al. 1982b; Day et al. 1983; Edward and Gilburn 2013;
336 Mérot et al. in press). Karyotypic specific survival differed across populations

337 suggesting that that population specific variation has accumulated in the
338 inversion. For example, ββ was the most common karyotype on Mölle wrack for
339 every population except Steninge (Figure 4). Instead, in Steninge, little variance
340 was seen between substrate combinations compared to other populations.

341 Our results also indicate that inversion-related size effects in *C. frigida* are
342 affected by the environment, particularly in males as females show little to no
343 effect of karyotype on size. Male fly inversion phenotypes from the same
344 population could vary two-fold for weight when grown on the same substrate (e.g.
345 Steninge population on Mölle substrate), and three-fold when comparing male
346 inversion phenotypes across all four growing substrates (e.g. Steninge, Figure 2).
347 Male size has a high heritability with virtually all the variance in size being
348 attributable to the chromosomal inversion system (Wilcockson et al. 1995). In all
349 of our treatment combinations, environmental effects never swamped the effect
350 of the inversion. Male αα were always the biggest, followed by αβ and ββ, which
351 is consistent with previous work (Figure 1a; e.g. Butlin et al. 1982a). Population
352 and substrate changed the magnitude of these differences as well as size
353 averages but never the pattern of the inversion size effects on males. Part of this
354 variation may be due to parental effects as the parents were reared in their
355 "home" substrate. Parental larval diet has been shown to influence offspring size
356 in *Drosophila melanogaster* (ex: Valtonen et al. 2012; Vijendravarma et al. 2010).
357 However, despite strong environment and inversion effects on male size, the
358 inversion, the population, and the environment exhibited negligible size effects in
359 females. Specifically, our data show much lower between inversion variation in

size and considerably less variation across environments for females. Thus, if maternal effects are present they are not influencing males and females in a similar manner. A similar difference in effect size between males and females has also been found in a common garden study on *C. frigida* grown on artificially mixed substrates containing in excess either the seaweed genus *Laminaria* or *Fucus* (Edward and Gilburn 2013). The study by Edward and Gilburn (2013) also found that flies grown on an excess of *Laminaria* showed greater among inversion size differences compared to *Fucus*, suggesting that the type of seaweed itself, or the associated microbiome of the seaweed, causes phenotypic differences in size. The wrackbed composition differs markedly along the populations studied, as a consequence of the declining salinity from the North Sea to the Baltic Sea (See Figure 1 and 2 in Wellenreuther et al. 2017) , and have likely been major selective environmental factors in modulating *C. frigida* populations. Development time, another trait known to be affected by inversion type, also showed significantly higher variation across male karyotypes, but exhibited less extreme environmentally induced effects compared to size, and consequently no significant GxE effects. These results together with previous studies (Butlin and Day 1984a; Day et al. 1980) underscore that the inversion related phenotype effects in *C. frigida* are significantly affected by sex, with male phenotypes being more labile compared to female phenotypes.

Interactions between genotypes and the environment are commonly observed in quantitative traits (Gupta and Lewontin 1982), particularly those associated with fitness (e.g. Fanara et al. 2006), and are thought to provide a potent force for

383 maintaining genetic variation (Fernández Iriarte and Hasson 2000; Gillespie and
384 Turelli 1989; Hedrick 1986). The habitat used by *C. frigida* is heterogeneous and
385 the resulting local population structure is similar to Levene's migration model of
386 subdivided populations, namely by a single pool of individuals that mate at
387 random and breed on discrete and ephemeral resources. Environmental
388 fluctuations in seaweed substrate composition likely favors the persistence of
389 alternative rearrangements (i.e. the α/β inversion) containing sets of co-adapted
390 alleles via balancing selection (Carroll et al. 2001; Filchak et al. 2000; Weinig
391 2000), with each set being optimal under different conditions (Dobzhansky 1970).
392 This hypothesis may account for the so called “supergene” idea (Schwander et
393 al. 2014) such as those determining mating types found in heterostylous plants
394 and wing mimicry polymorphisms found in *Heliconius* butterflies (Thompson and
395 Jiggins 2014). Balancing selection resulting from seasonal and stochastic
396 temporal and spatial wrackbed changes may thus be a significant contributor to
397 maintaining the fitness related inversion variation in size. Other examples for
398 selection on fitness trade-offs come from introduced apple *Malus pumila* and
399 native hawthorn *Crataegus* spp. hosts which has led to divergence in phenology
400 and thermal physiology in the true fruit fly *Rhagoletis pomonella* (Filchak et al.
401 2000).

402 Balancing selection has also been implicated in the maintenance of other
403 inversion polymorphisms, but whether balancing selection can maintain genetic
404 polymorphisms over long time scales is still debated (Bernatchez 2016).
405 Theoretical studies indicate that balancing selection may primarily be a short-

term process because the genetic load that it creates then has a negative feedback that hinders the long-term maintenance of adaptive alleles (Fijarczyk and Babik 2015). Furthermore, rapid environmental changes may cause dramatic allele frequency shifts that could cause the loss of formerly stable polymorphisms (Fijarczyk and Babik 2015). Despite this, recent studies have provided some evidence that balancing selection can operate even over longer time scales. Lindtke et al. (2017) investigated the inversion related cryptic colour polymorphism in *Timema cristinae* walking stick insects using a comprehensive dataset of natural populations and population genetic analyses. They were able to demonstrate that inversion colour variation has likely been maintained over extended time periods by balancing selection, which has previously been thought to be only possible over short time frames. Balancing selection (via spatially variable selection) on chromsomal inversions has been widely demonstrated in *Drosophila* species (Wellenreuther and Bernatchez 2018). The most well studied of these is the In(3R)Payne inversion in *Drosophila melanogaster*, which shows stable parallel clines pertaining to latitude on 3 continents (Kapun et al. 2016). In *Drosophila pseudoobscura* many different arrangements on the third chromosome form clines across the Southwestern United States that are maintained by a migration selection balance between different environmental niches (Schaeffer 2008).

In conclusion, our results indicate that the inversion in *C. frigida* likely evolves via a combination of local mutation, GxE effects, and differential fitness of karyotypes in heterogeneous environments. Future work should apply high-

429 resolution genome-wide approaches that target the regions inside the inversion
430 to identify the true genic targets of varying selection. This approach would also
431 elucidate which genes inside the inversion are linked to these large size effects in
432 males, and how the environment has shaped the differentiation between these
433 genes along gradients.

434

435 Acknowledgements

436 MW would like to thank Roger Butlin and Andre Gilburn for helpful advice
437 regarding the molecular inversion determination and rearing techniques. EB
438 would like to thank Fabian Roger for helpful advice regarding the figures. Helena
439 Westerdahl and Jan Åke Nilsson gave some helpful comments on earlier
440 versions of this work. The work was supported by a grant from the Crafoord
441 Foundation, from Entomological Foundation in Lund and from the Swedish
442 Research Vetenskapsrådet Council (2012-3996) to MW. EB was supported by a
443 Marie Skłodowska-Curie fellowship 704920 — ADAPTIVE INVERSIONS —
444 H2020-MSCA-IF-2015.

445

446 Conflict of Interest

447 The authors declare that they have no conflict of interest.

448

449 Data Archiving

All raw phenotypic data and karyotype frequencies will be deposited on Dryad upon acceptance.

References

Aziz JB (1975) Investigations into chromosomes 1, 2 and 3 of Coelopa frigida (Fab.). Thesis,

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4 arXiv preprint arXiv:14065823

Bernatchez L (2016) On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes Journal of Fish Biology 89:2519-2556

Bland JM, Altman DG (1995) Multiple significance tests: the Bonferroni method Bmj 310:170

Butlin RK (1983) The maintenance of an inversion polymorphism in Coelopa frigida. University of Nottingham

Butlin RK, Collins P, Skevington S, Day T (1982a) Genetic variation at the alcohol dehydrogenase locus in natural populations of the seaweed fly, *Coelopa frigida* Heredity 48:45-55

Butlin RK, Collins PM, Skevington SJ, Day TH (1982b) Genetic-Variation at the Alcohol-Dehydrogenase Locus in Natural-Populations of the Seaweed Fly, Coelopa-Frigida Heredity 48:45-55 doi:DOI 10.1038/hdy.1982.5

Butlin RK, Day TH (1984a) The effect of larval competition on development time and adult size in the seaweed fly, *Coelopa frigida* Oecologia 63:122-127

Butlin RK, Day TH (1984b) The Effect of Larval Competition on Development Time and Adult Size in the Seaweed Fly, Coelopa-Frigida Oecologia 63:122-127 doi:Doi 10.1007/Bf00379793

Butlin RK, Day TH (1989) Environmental correlates of inversion frequencies in natural populations of seaweed flies (*Coelopa frigida*) Heredity 62:223-232

Butlin RK, Read IL, Day TH (1982c) The effects of a chromosomal inversion on adult size and male mating success in the seaweed fly, *Coelopa frigida* Heredity 49:51-62

Carreira VP, Soto IM, Hasson E, Fanara JJ (2006) Patterns of variation in wing morphology in the cactophilic Drosophila buzzatii and its sibling D. koepferae J Evol Biol 19:1275-1282 doi:10.1111/j.1420-9101.2005.01078.x

Carroll SP, Dingle H, Famula TR, Fox CW (2001) Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, Jadera haematoloma Genetica 112:257-272

Collins PM (1978) Studies on genetic polymorphism in *Coelopa frigida*. The Genetical Society of Great Britain,

Cullen SJ, Young AM, Day TH (1987) Dietary requirements of seaweed flies (Coelopa frigida) Estuarine, Coastal and Shelf Science 24:701-710

Day T, Dobson T, Hillier P, Parkin D, Clarke B (1982a) Associations of enzymic and chromosomal polymorphisms in the seaweed fly, Coelopa frigida Heredity 48:35-44

Day TH, Buckley PA (1980) Alcohol dehydrogenase polymorphism in the seaweed fly, *Coelopa frigida* Biochem Genet 18:727-742 doi:10.1007/bf00484589

Day TH, Dawe C, Dobson T, Hillier PC (1983) A chromosomal inversion polymorphism in Scandinavian populations of the seaweed fly, Coelopa frigida Hereditas 99:135-145 doi:10.1111/j.1601-5223.1983.tb00738.x

Day TH, Dobson T, Hillier PC, Parkin DT, Clarke B (1982b) Associations of enzymic and chromosomal polymorphisms in the seaweed fly, Coelopa frigida Heredity 48:35-44

Day TH, Dobson T, Hillier PC, Parkin DTa, Clarke B (1980) Different rates of development associated with the alcohol dehydrogenase locus in the seaweed fly, Coelopa frigida Heredity 44:321-326

Dobzhansky T (1948) Genetics of natural populations. XVI. Altitudinal and seasonal changes produced by natural selection in certain populations of *Drosophila pseudoobscura* and *Drosophila persimilis* Genetics 33:158

Dobzhansky TG (1970) Genetics of the evolutionary process. Columbia University Press, New York

Edward DA, Gilburn AS (2013) Male-specific genotype by environment interactions influence viability selection acting on a sexually selected inversion system in the seaweed fly, *Coelopa frigida* Evolution 67:295-302

Endler JA (1977) Geographic variation, speciation, and clines. Princeton University Press,

Falconer D (1990) Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance Genetics Research 56:57-70

Fanara J, Folguera G, Iriarte PF, Mensch J, Hasson E (2006) Genotype by environment interactions in viability and developmental time in populations of cactophilic Drosophila Journal of Evolutionary Biology 19:900-908

Fernández Iriarte P, Hasson E (2000) The role of the use of different host plants in the maintenance of the inversion polymorphism in the cactophilic Drosophila buzzatii Evolution 54:1295-1302

Fijarczyk A, Babik W (2015) Detecting balancing selection in genomes: limits and prospects Molecular Ecology 24:3529-3545

541 Filchak KE, Roethele JB, Feder JL (2000) Natural selection and sympatric
542 divergence in the apple maggot Rhagoletis pomonella Nature 407:739-
543 742

544 Fox J, Weisberg S (2011) An R Companion to Applied Regression. 2 edn. SAGE
545 Publications, Thousand Oaks, California

546 Fuller RA (2003) Factors influencing foraging decisions in ruddy turnstones
547 Arenaria interpres (L.). Durham University

548 Gilburn AS, Day TH (1994) Sexual dimorphism, sexual selection and the α β
549 chromosomal inversion polymorphism in the seaweed fly, *Coelopa frigida*
550 Proceeding of the Royal Society of Biological Sciences 257:303-309

551 Gilburn AS, Foster SP, Day TH (1992) Female mating preference for large size in
552 *Coelopa frigida* (seaweed fly) Heredity 69:209-216

553 Gillespie JH, Turelli M (1989) Genotype-environment interactions and the
554 maintenance of polygenic variation Genetics 121:129-138

555 Gupta AP, Lewontin R (1982) A study of reaction norms in natural populations of
556 Drosophila pseudoobscura Evolution 36:934-948

557 Hedrick PW (1986) Genetic polymorphism in heterogeneous environments: a
558 decade later Annual review of ecology and systematics 17:535-566

559 Hijmans RJ, van Etten J (2014) raster: Geographic data analysis and modeling R
560 package version 2

561 Hoekstra HE, Coyne JA (2007) The locus of evolution: evo devo and the genetics
562 of adaptation Evolution 61:995-1016 doi:10.1111/j.1558-
563 5646.2007.00105.x

564 Hoffmann AA, Rieseberg LH (2008) Revisiting the impact of inversions in
565 evolution: from population genetic markers to drivers of adaptive shifts and
566 speciation? Annual Review of Ecology, Evolution, and Systematics 39:21-
567 42 doi:doi:10.1146/annurev.ecolsys.39.110707.173532

568 Kapun M, Fabian DK, Goudet J, Flatt T (2016) Genomic Evidence for Adaptive
569 Inversion Clines in Drosophila melanogaster Mol Biol Evol 33:1317-1336
570 doi:10.1093/molbev/msw016

571 Kirkpatrick M (2010) How and why chromosome inversions evolve PLoS Biol
572 8:e1000501 doi:10.1371/journal.pbio.1000501

573 Kirkpatrick M, Barton N (2006) Chromosome inversions, local adaptation and
574 speciation Genetics 173:419-434

575 Krishnamoorthy K, Lee M (2014) Improved tests for the equality of normal
576 coefficients of variation Computational Statistics 29:215-232

577 Leggett M, Wilcockson R, Day T, Phillips D, Arthur W (1996) The genetic effects
578 of competition in seaweed flies Biol J Linn Soc 57:1-11

579 Lindtke D et al. (2017) Long-term balancing selection on chromosomal variants
580 associated with crypsis in a stick insect Molecular Ecology 26:xxx-xxx
581 doi:10.1111/mec.14280

582 Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits vol 1.
583 Sinauer Sunderland, MA,

584 Maier MJ (2014) DirichletReg: Dirichlet regression for compositional data in R
585 Research Report Series / Department of Statistics and Mathematics WU
586 Vienna University of Economics and Business:125

587 Marwick B, Krishnamoorthy K (2016) cvequality: Tests for the Equality of
588 Coefficients of Variation from Multiple Groups., R package version 0.1.1.
589 edn.,

590 Mcalpine DK (1991) Review of the Australian Kelp Flies (Diptera, Coelopidae)
591 Syst Entomol 16:29-84 doi:DOI 10.1111/j.1365-3113.1991.tb00573.x

592 Mérot C, Berdan EL, Babin C, Normandeau E, Wellenreuther M, Bernatchez L (in
593 press) Inter-continental karyotype-environment parallelism supports a role
594 for a chromosomal inversion in local adaptation in a seaweed fly
595 Proccedings of the Royal Society B

596 R Development Core Team R (2015) R: A language and environment for
597 statistical computing vol 1. R Foundation for Statistical Computing. R
598 Foundation for Statistical Computing. doi:10.1007/978-3-540-74686-7

599 Reinhardt JA, Kolaczkowski B, Jones CD, Begun DJ, Kern AD (2014) Parallel
600 geographic variation in Drosophila melanogaster Genetics 197:361-373

601 Savolainen O, Lascoux M, Merila J (2013) Ecological genomics of local
602 adaptation Nature Review Genetics 14:807-820 doi:10.1038/nrg3522

603 Sbrocco EJ, Barber PH (2013) MARSPEC: ocean climate layers for marine
604 spatial ecology Ecology 94:979-979

605 Schaeffer SW (2008) Selection in Heterogeneous Environments Maintains the
606 Gene Arrangement Polymorphism of Drosophila Pseudoobscura Evolution
607 62:3082-3099 doi:10.1111/j.1558-5646.2008.00504.x

608 Schrider DR, Hahn MW, Begun DJ (2016) Parallel evolution of copy-number
609 variation across continents in Drosophila melanogaster Molecular biology
610 and evolution 33:1308-1316

611 Schwander T, Libbrecht R, Keller L (2014) Supergenes and complex phenotypes
612 Current Biology 24:288-294 doi:10.1016/j.cub.2014.01.056

613 Storz JF (2002) Contrasting patterns of divergence in quantitative traits and
614 neutral DNA markers: analysis of clinal variation Mol Ecol 11:2537-2551

615 Sturtevant AH (1921) A case of rearrangement of genes in *Drosophila*
616 Proceedings of The National Academy of Science of the united States of
617 America 7:235–237

618 Summers RW, Smith S, Nicoll M, Atkinson NK (1990) Tidal and Sexual
619 Differences in the Diet of Purple Sandpipers Calidris-Maritima in Scotland
620 Bird Study 37:187-194

621 Thompson M, Jiggins C (2014) Supergenes and their role in evolution Heredity
622 113:1-8

623 Tigano A, Friesen VL (2016) Genomics of local adaptation with gene flow
624 Molecular ecology

625 Ungerer MC, Halldorsdottir SS, Purugganan MD, Mackay TF (2003) Genotype-
626 environment interactions at quantitative trait loci affecting inflorescence
627 development in Arabidopsis thaliana Genetics 165:353-365

628 Valtonen TM, Kangassalo K, Polkki M, Rantala MJ (2012) Transgenerational
629 Effects of Parental Larval Diet on Offspring Development Time, Adult
630 Body Size and Pathogen Resistance in Drosophila melanogaster Plos
631 One 7 doi:ARTN e31611

632 10.1371/journal.pone.0031611

633 Via S, Lande S (1985) Genotype–environment interaction and the evolution of
634 phenotypic plasticity Evolution 39:505-522

635 Vijendravarma RK, Narasimha S, Kawecki TJ (2010) Effects of parental larval
636 diet on egg size and offspring traits in Drosophila Biol Lett 6:238-241
637 doi:10.1098/rsbl.2009.0754

638 Weinig C (2000) Plasticity versus canalization: population differences in the
639 timing of shade-avoidance responses Evolution 54:441-451

640 Wellenreuther M, Bernatchez L (2018) Eco-Evolutionary Genomics of
641 Chromosomal Inversions Trends Ecol Evol 33:427-440
642 doi:10.1016/j.tree.2018.04.002

643 Wellenreuther M, Hansson B (2016) Detecting polygenic evolution: problems,
644 pitfalls, and promises Trends in Genetics 32:155–164
645 doi:10.1016/j.tig.2015.12.004

646 Wellenreuther M, Rosenquist H, Jaksons P, Larson W (2017) Local adaptation
647 along an environmental cline in a species with an inversion polymorphism
648 J Evol Biol 30:1068-1077

649 Wilcockson R, Crean C, Day T (1995) Heritability of a Sexually Selected
650 Character Expressed in Both Sexes Nature 374:158-159

651 Yeaman S (2013) Genomic rearrangements and the evolution of clusters of
652 locally adaptive loci Proceedings of the National Academy of Sciences
653 doi:10.1073/pnas.1219381110

654

Tables

Table 1. Spatial coordinates (WGS 1984 DD), sea surface salinity (PSU), mean air temperature, inversion frequency, and wrackbed composition of the study sites.

	Coordinates (WGS84, DD)		Sea surface salinity (PSU)[a]		Air Temperature (°C)[b]	Inversion frequency[c]	Wrackbed composition			
	Latitude	Longitude	Mean annual	Annual SD	Mean annual	Frequency of the α inversion	Percentage *Fucus spp.*	Percentage Kelp[d]	Percentage red algae[e]	Percentage eelgrass[f]
Steninge	56.77	12.63	15.79	16.62	7.5	0.49	50	20	30	0
Mölle	56.28	12.49	15.38	16.88	7.8	0.42	30	30	30	10
Kämpinge	55.4	12.98	12.82	11.74	8.1	0.35	30	0	30	40
Käseberga	55.38	14.06	9.91	5.4	8.0	0.34	30	0	50	20

[a] Data source: Sbrocco & Barber (2013), accessed on 1 November 2016.
[b] Data source: WorldClim database (1.4) , accessed in May 2018
[c] Day et al. (1983).
[d] *Saccharina* and *Laminaria spp.*
[e] *Furcellaria lumbricalis*
[f] *Zostera marina*

Table 2. Best models for adult weight and development time. An X indicates that a main effect or interaction was present in the best model.

	Main Effects				2-way interactions						3-way interactions
	Karyotype	Population	Sex	Substrate	Karyotype:Population	Karyotype:Sex	Karyotype:Substrate	Population:Sex	Population:Substrate	Sex:Substrate	Karyotype:Population:Substrate
Weight	x	x	x	x	x	x	x	x	x		x
Development Time	x	x	x	x		x					

Table 3. Results from Krishnamoorthy and Lee's (2014) modified signed-likelihood ratio test (MS-LRT) looking to see if the coefficient of variation is different between males and females. Bolded p-values are significant at the P< 0.0001 level after a Bonferroni correction.

Trait	MS-LRT Statistic	P-value
Weight	79.22	**0**
Thorax Width	83.79	**0**
Leg Length	61.64	**4.11E-15**
Wing Length	118.05	**0**
Development Time	22.09	**2.61E-06**

Titles and legends to figures

Figure 1: A. Picture of an αα, αβ, and ββ male showcasing the size differential. B. Map of *C. frigida* fly populations used in this study. Base map from d-maps (http://d-maps.com/carte.php?num_car=23102).

Figure 2: Boxplot of weight across populations (listed at Top), substrate (listed on x-axis), sex (indicated by top (male) and bottom (female) charts), and karyotype (indicated by color, red - αα, blue - αβ, and green-ββ). Outliers are not shown for clarity. N for each population x sex combination (from left to right for each karyotype and substrate as listed in the figure): Kåseberga Male: 10, 19, 5, 2, 11, 6, 5, 9, 10, 2, 9, 6, Kämpinge Male: 3, 20, 15, 1, 9, 4, 3, 13, 19, 1, 21, 6, Mölle Male: 10, 14, 1, 6, 8, 13, 6, 12, 13, 2, 6, 10, Steninge Male: 7, 17, 7, 0, 3, 2, 5, 15, 5, 7, 16, 14, Kåseberga Female: 7, 24, 11, 2, 3, 6, 4, 12, 18, 5, 13, 5, Kämpinge Female: 1, 12, 11, 0, 5, 4, 0, 13, 18, 3, 17, 9, Mölle Female: 4, 9, 4, 4, 10, 4, 6, 6, 5, 4, 3, 8, Steninge Female: 8, 21, 8, 2, 5, 4, 8, 19, 6, 7, 21, 7.

Figure 3: Boxplot of development time across populations (listed at Top), substrate (listed on x-axis), sex (indicated by top (male) and bottom (female) charts), and karyotype (indicated by color, black - αα, grey - αβ, and white-ββ). Outliers are not shown for clarity. N for each population x sex combination (from left to right for each karyotype and substrate as listed in the figure): Kåseberga Male: 10, 19, 5, 2, 11, 6, 5, 9, 10, 2, 9, 6, Kämpinge Male: 3, 20, 15, 1, 9, 4, 3,

13, 19, 1, 21, 6, Mölle Male: 10, 14, 13, 1, 6, 8, 6, 12, 13, 2, 6, 10, Steninge Male: 7, 17, 7, 0, 3, 2, 5, 15, 5, 7, 16, 14, Kåseberga Female: 7, 24, 11, 2, 3, 6, 4, 12, 18, 5, 13, 5, Kämpinge Female: 1, 12, 11, 0, 5, 4, 0, 13, 18, 3, 17, 9, Mölle Female: 4, 9, 4, 4, 10, 4, 6, 6, 5, 4, 3, 8, Steninge Female: 8, 21, 8, 2, 5, 4, 8, 19, 6, 7, 21, 7.

Figure 4: Box plot of frequencies of different karyotypes (black - αα, grey - αβ, and white-ββ) across populations (listed at the top of each graph) and substrates (listed on the x-axis).

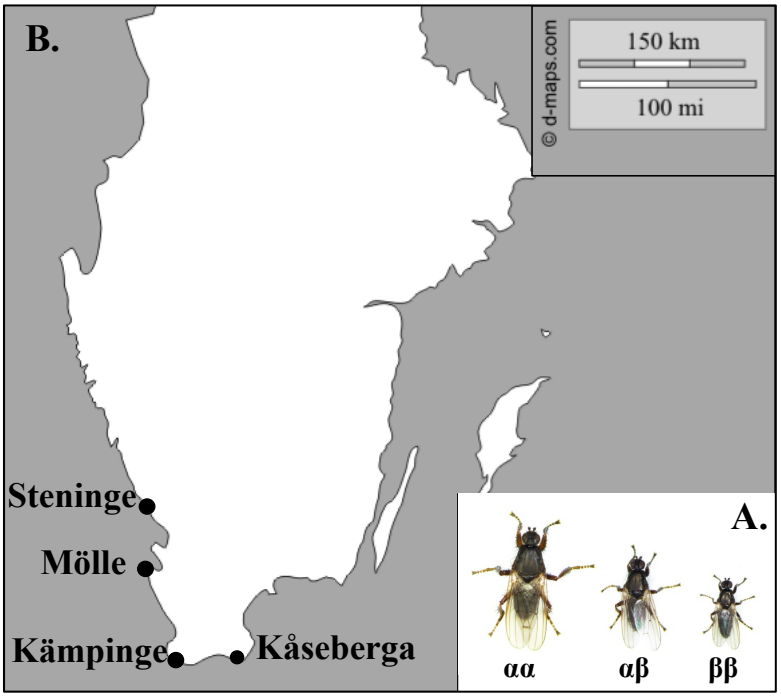
B.
© d-maps.com
150 km
100 mi
Steninge
Mölle
Kämpinge
Kåseberga
A.
αα
αβ
ββ

Figure 1

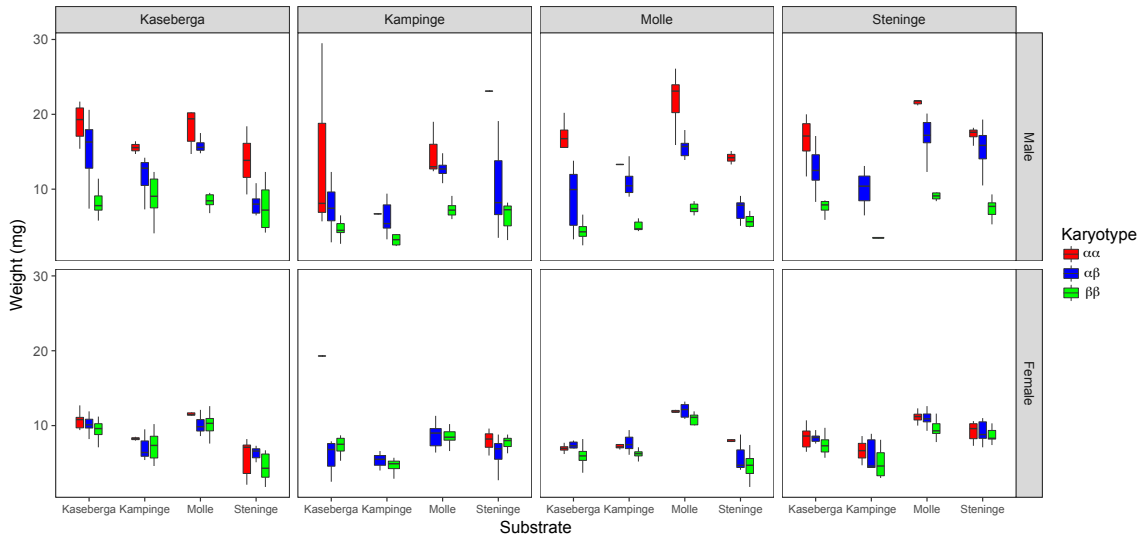
Kaseberga
Kampinge
Molle
Steninge
Male
Female
Weight (mg)
30
20
10
Kaseberga Kampinge Molle Steninge
Substrate
Karyotype
αα
αβ
ββ

Figure 2

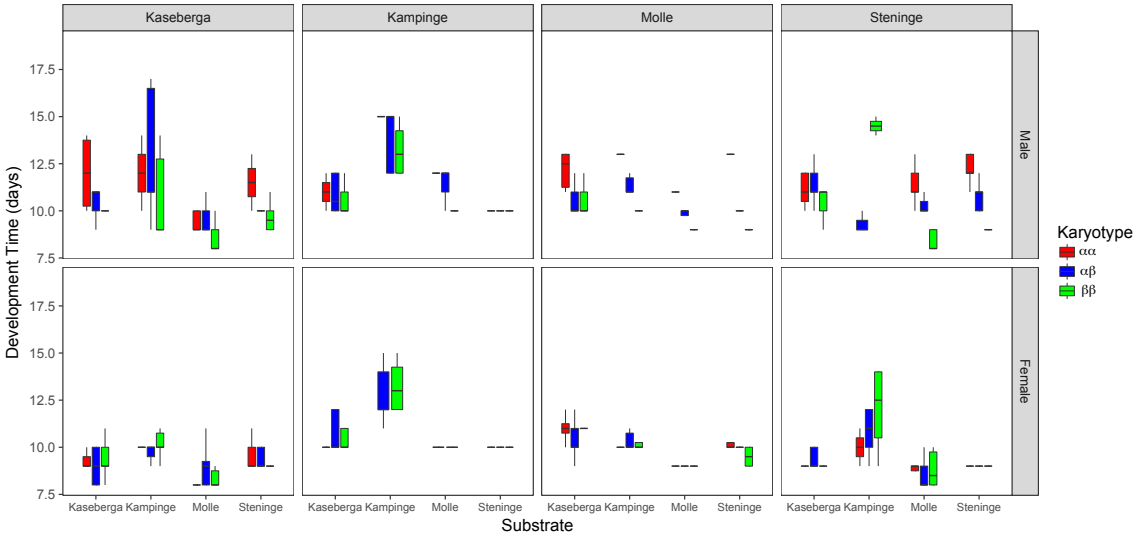
Kaseberga
Kampinge
Molle
Steninge
Male
Female
Development Time (days)
17.5
15.0
12.5
10.0
7.5
Kaseberga Kampinge Molle Steninge
Substrate
Karyotype
ααα
αβ
ββ

Figure 3

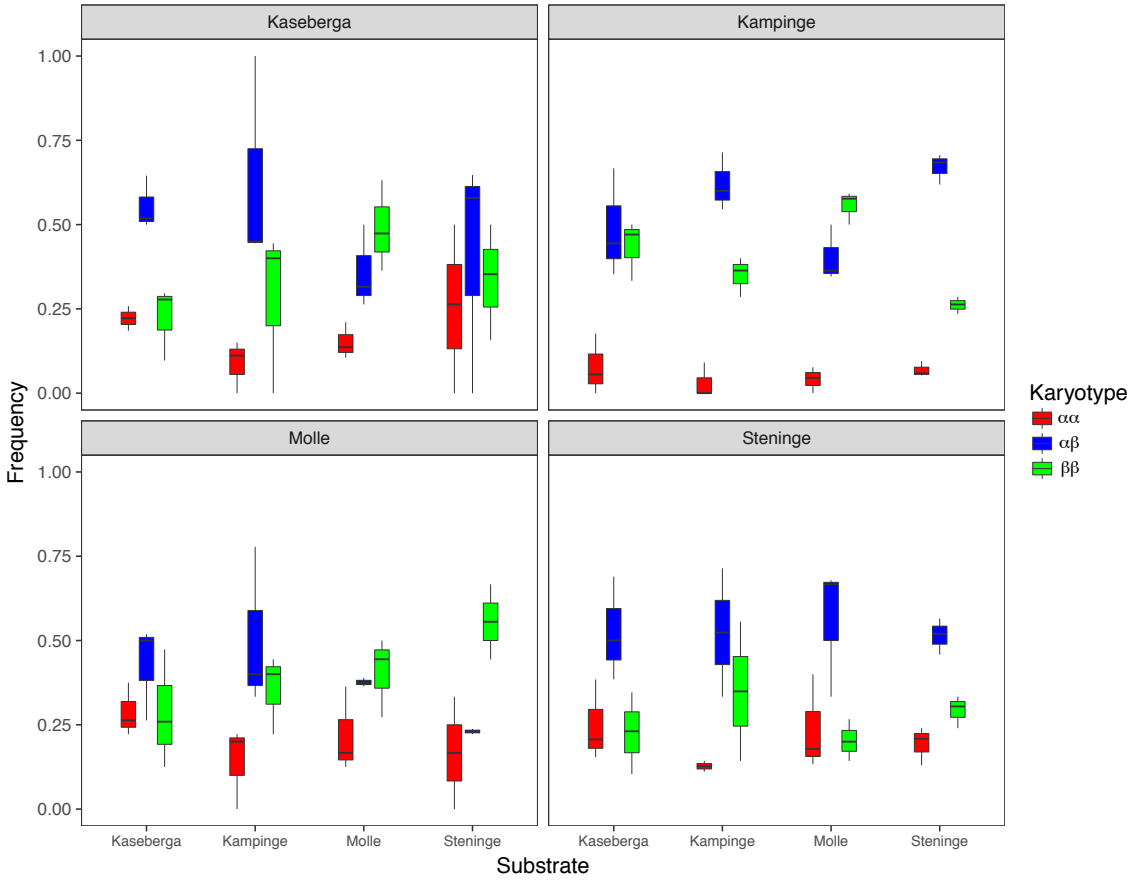
Kaseberga
Kampinge
Molle
Steninge
Frequency
1.00
0.75
0.50
0.25
0.00
Kaseberga
Kampinge
Molle
Steninge
Substrate
Karyotype
αα
αβ
ββ

Figure 4

Supplemental Material

Table S1. Pearson correlations of morphological factors.

	Adult Weight	Thorax Width	Wing Length	Leg Length
Weight	1	0.8306678	0.8850752	0.7786367
Thorax Width	0.8306678	1	0.8452453	0.7628986
Wing length	0.8850752	0.8452453	1	0.865498
Leg Length	0.7786367	0.7628986	0.865498	1

Table S2. ANOVA tables for the full models adult weight (A) and development time (B). Bolded terms are included in the final models.

A.

Term	DF	Sum of Squares	Mean Square	F-Value
Population	**3**	**201.08**	**67.03**	**13.6683**
Substrate	**3**	**327.74**	**109.25**	**22.2781**
Sex	**1**	**1559.27**	**1559.27**	**317.9702**
Karyotype	**2**	**2589.74**	**1294.87**	**264.0532**
Population:Substrate	**9**	**185.65**	**20.63**	**4.2065**
Population:Sex	**3**	**422.75**	**140.92**	**28.7363**
Population:Karyotype	**6**	**228.57**	**38.09**	**7.7683**
Substrate:Sex	3	18.59	6.2	1.264
Substrate:Karyotype	**6**	**116.87**	**19.48**	**3.9719**
Sex:Karyotype	**2**	**1406.79**	**703.4**	**143.4383**
Population:Substrate:Sex	9	64.31	7.15	1.4572
Population:Substrate:Karyotype	**18**	**220.67**	**12.26**	**2.5**
Population:Sex:Karyotype	6	36.49	6.08	1.2401
Substrate:Sex:Karyotype	6	53.67	8.95	1.8242

B.

Term	DF	Sum of Squares	Mean Square	F-Value
Population	**3**	**11.039**	**3.6796**	**3.6796**
Substrate	**3**	**35.277**	**11.759**	**11.759**
Sex	**1**	**20.778**	**20.7776**	**20.7776**
Karyotype	**2**	**6.958**	**3.4792**	**3.4792**
Population:Substrate	9	9.476	1.0528	1.0528
Population:Sex	3	6.46	2.1533	2.1533
Population:Karyotype	6	0.461	0.0769	0.0769
Substrate:Sex	3	0.598	0.1994	0.1994
Substrate:Karyotype	6	1.639	0.2732	0.2732
Sex:Karyotype	**2**	**6.725**	**3.3625**	**3.3625**
Population:Substrate:Sex	9	3.591	0.399	0.399
Population:Substrate:Karyotype	18	4.861	0.2701	0.2701
Population:Sex:Karyotype	6	0.263	0.0438	0.0438
Substrate:Sex:Karyotype	6	0.534	0.0889	0.0889

Figure S2: Boxplot of thorax width across populations (listed at Top), substrate (listed on x-axis), sex (indicated by top (male) and bottom (female) charts), and karyotype (indicated by color, red - αα, blue - αβ, and green-ββ). Outliers are not shown for clarity.

Figure S3: Boxplot of leg length across populations (listed at Top), substrate (listed on x-axis), sex (indicated by top (male) and bottom (female) charts), and karyotype (indicated by color, red - αα, blue - αβ, and green-ββ). Outliers are not shown for clarity.

Figure S4: Boxplot of wing length across populations (listed at Top), substrate (listed on x-axis), sex (indicated by top (male) and bottom (female) charts), and karyotype (indicated by color, red - αα, blue - αβ, and green-ββ). Outliers are not shown for clarity.

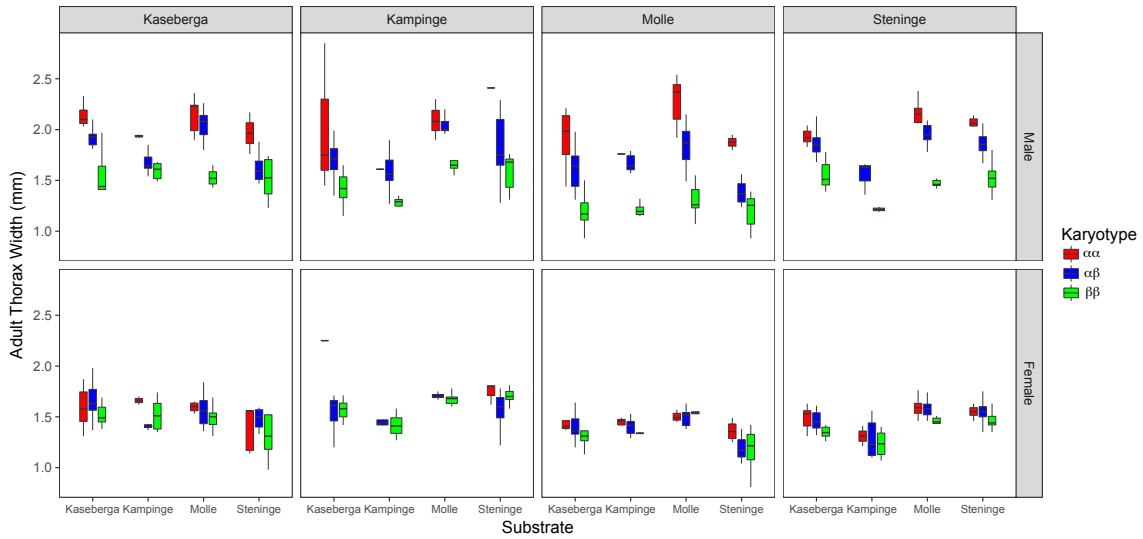
Kaseberga
Kampinge
Molle
Steninge
Male
Female
Adult Thorax Width (mm)
2.5
2.0
1.5
1.0
Kaseberga Kampinge Molle Steninge
Substrate
Karyotype
ααα
αβ
ββ

Figure S2

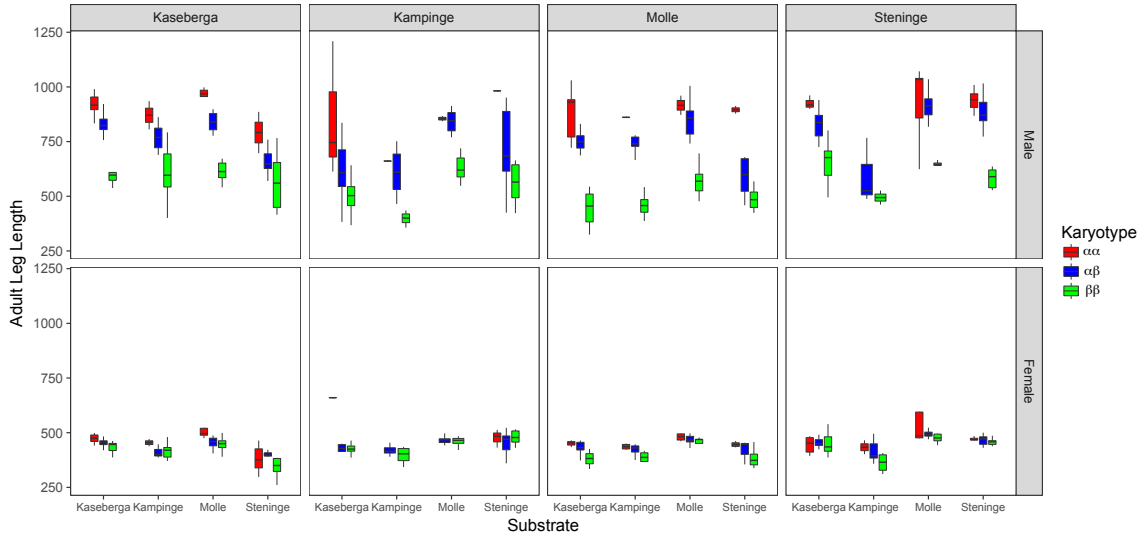
Kaseberga
Kampinge
Molle
Steninge
Male
Female
Adult Leg Length
1250
1000
750
500
250
Substrate
Karyotype
αα
αβ
ββ

Figure S3

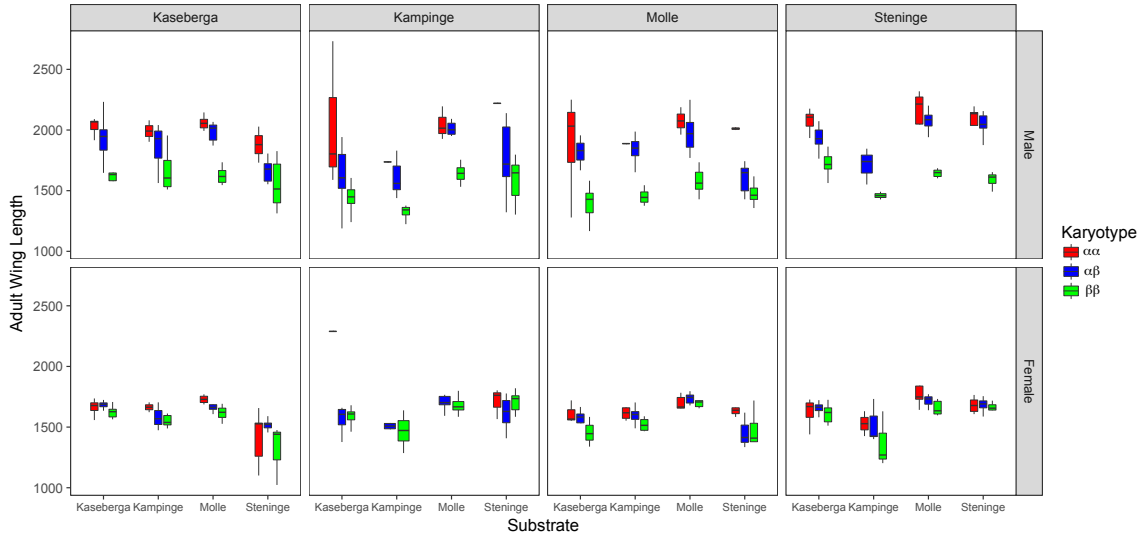
Kaseberga
Kampinge
Molle
Steninge
Male
Female
Adult Wing Length
2500
2000
1500
1000
Kaseberga Kampinge Molle Steninge
Substrate
Karyotype
αα
αβ
ββ

Figure S4