UNIVERSITEIT GENT

Faculty of sciences

Department of biology

Terrestrial ecology unit

Academic year 2015-2016

Eco-evolutionary responses along an experimental dispersal front, using *Tetranychus urticae* as a model species

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Master's dissertation submitted to obtain the degree of Master of Science in Biology

Introduction

Human induced climate change is predicted to increasingly threaten the survival of many species (Deutsch, Tewksbury, Huey *et al.*, 2008; Harvell, Mitchell, Ward *et al.*, 2002; Thomas, Cameron, Green *et al.*, 2004; Thuiller, Lavorel, Araujo *et al.*, 2005). One of the major ways species can cope with this increasing pressure is by shifting their geographical range (Deutsch *et al.*, 2008; Mustin, Benton, Dytham *et al.*, 2009; Thomas *et al.*, 2004) through dispersal, which itself can be described as any kind of movement of individuals or propagules with potential consequences for gene flow across space (Ronce, 2007). Consequently, the rate at which species shift their range is expected to accelerate during periods of climate change (Mustin *et al.*, 2009). The process of range shifting therefore distinguishes itself from many other ecological and evolutionary processes, which are studied in the context of a stable or equilibrium condition in terms of spatial distribution, as there is a continuous (and usually directional) displacement of individuals in terms of spatial distribution (Burton, Phillips & Travis, 2010; Phillips, Brown & Shine, 2010b). Climate-induced geographical range shifts have already been observed in many species from a broad taxonomical spectrum (Hickling, Roy, Hill *et al.*, 2006). Especially insects are very prone to suffer the effects of climate change, causing either a range retraction (Thomas, Franco & Hill, 2006) or expansion (Parmesan, Ryrholm, Stefanescu *et al.*, 1999), and are predicted to be severely affected in the future (Deutsch *et al.*, 2008).

As dispersive individuals will be prone to leave the original home patch, and philopatric (non-dispersive) ones will be more likely to remain, the distribution of species along the dispersal front will be shaped by spatial sorting related to dispersal ability, with dispersive individuals accumulating at the edge. The interplay between this spatial sorting and assortative mating between individuals that are alike in terms of dispersal ability leads to even more dispersive individuals (Olympic village effect). (Kubisch, Hovestadt & Poethke, 2010; Phillips, Brown, Travis *et al.*, 2008; Shine, Brown & Phillips, 2011) Consequentially, dispersal kernels are expected to both show a higher mean dispersal distance and more leptokurtic at the dispersal front (Phillips *et al.*, 2008). This runaway selection on dispersal related traits is expected to occur as long the species continues to shift its range (Phillips *et al.*, 2010b). In case individuals suffer from strong fitness disadvantages at very low densities (Allee effects), selection on dispersal traits can be severely limited as the strongest dispersers suffer the consequences of ending up in very low density regions at the edge of the dispersal front (Travis & Dytham, 2002). When species are no longer able to continue shifting their range (due to for example environmental constraints), selection against dispersal traits is to be expected, as strong dispersers tend to end up in non-inhabitable regions (Simmons & Thomas, 2004).

The process of dispersal (be it in the context of range expansion or otherwise) is however not a random process, in the sense that the dispersive individuals are not a random subset of the population, but rather those individuals for which benefits of dispersal will outweigh the associated costs during the different phases of dispersal (departure, transience and settlement) (Bonte, Van Dyck, Bullock *et al.*, 2012; Clobert, Le Galliard, Cote *et al.*, 2009; Travis, Mustin, Barton *et al.*, 2012). For instance dispersive individuals of spider mites have been demonstrated to significantly increase their fitness under low density conditions compared to high density conditions, whereas philopatric (non-dispersive) individuals did not have a significantly different fitness under both conditions (Bonte, De Roissart, Wybouw *et al.*, 2014). The adaptive responses at the dispersal front are not always straightforward and are dependent on both the costs associated with leaving or remaining in the homepatch. For instance when dispersal has high energetic costs or is physically very demanding, only individuals with a high body mass or investment in movement related traits (wing size, leg length, etc.) may be able to disperse successfully (Bonte *et al.*, 2012; Chuang & Peterson, 2016). However smaller individuals may as well be more prone to suffer the consequences of intraspecific competition, and therefore more likely to leave the homepatch under high competition conditions, even though dispersal costs may be more severe for them compared to large individuals (Lawrence, 1987; Lena, Clobert, de Fraipont *et al.*, 1998). Differences in dispersal related traits between individuals from the edge and core of a dispersal front have regularly been observed, with the example of the invasive cane toad (Rhinella marina) in Australia one of the most extensively described cases. Cane toads at the edge of the dispersal front have been found to have longer legs (Phillips, Brown, Webb *et al.*, 2006), a larger body size (Phillips, Brown & Shine, 2010a), and to display more frequent dispersal behaviour, and over longer distances (Alford, Brown, Schwarzkopf *et al.*, 2009; Lindstroem, Brown, Sisson *et al.*, 2013). Morphological adaptations linked to dispersal behaviour at dispersal fronts have been regularly recorded in arthropods as well. For instance morphological adaptations towards increased flight capability were recorded in butterflies (Hill, Thomas & Blakeley, 1999) and damselflies (Therry, Bonte & Stoks, 2015) and larger body size has been found in beetles (Laparie, Renault, Lebouvier *et al.*, 2013). Apart from morphological adaptations for increased dispersal capability, behavioural adaptations towards more frequent or earlier dispersal have been recorded for instance in spider mites (Van Petegem, Boeye, Stoks *et al.*, 2015).

Phenotypic variation between dispersive and philopatric individuals is often not limited to traits related directly to dispersal behaviour as dispersive individuals enter novel environments in which different phenotypes may thrive compared to the core demes. (Bowler & Benton, 2005; Clobert *et al.*, 2009; Travis *et al.*, 2012) Dispersal fronts are typically characterized by low density condition, resulting in a selective advantage for fast reproducing individuals (r-

selection), whereas high intraspecific competition in the core may lead to high investment in competitive ability (K-selection) (Chuang *et al.*, 2016; Reznick, Bryant & Bashey, 2002). Adaptation towards faster sexual maturation (Amundsen, Salonen, Niva et al., 2012; Sanford, Holzman, Haney et al., 2006), increased reproductive output (Amundsen *et al.*, 2012) and increased investment in reproductive tissue (Ling, Johnson, Frusher *et al.*, 2008) were recorded at the edge of species' distributions. Behavioural traits such as aggression and a reduced fear to try novel food sources may be advantageous for a productivity oriented life history strategy (Cole & Quinn, 2012) to accommodate increased feeding rates at the edge of the dispersal front (Brown, Kelehear & Shine, 2013). Consequentially, spatial selection acting along during range expansion is expected to lead to accumulation of dispersive and fast reproducing individuals at the edge. Furthermore, it is expected that limitations in allocation of resources and energy will lead to trade-offs along the dispersal front (Chuang *et al.*, 2016). Investment in dispersal ability and faster population growth at the edge are expected to be traded off against competitive ability compared to the core populations (Burton *et al.*, 2010). For instance trade-offs between fast reproduction and longevity (Amundsen *et al.*, 2012) and with immune system (Brown, Shilton, Phillips *et al.*, 2007) have been detected at the edge of dispersal fronts. In some species, a dispersal-reproduction trade-off has been found (Mole & Zera, 1993; Zhang, Wu, Wyckhuys *et al.*, 2009), which may limit selection for faster reproduction at the edge of the dispersal front. However this trend is not universal, as in other species no indication for dispersal-reproduction trade-offs has been found, and increased dispersal and reproduction are argued to come at the cost of increased feeding (Hanski, Saastamoinen & Ovaskainen, 2006; Therry *et al.*, 2015). In their recent review paper concerning expanding populations, Chang and Peterson illustrate the multitude and broad nature trait differences observed along dispersal fronts (Chuang *et al.*, 2016).

Apart from the deterministic processes (as discussed in the sections above), stochastic process can play an important role as well in shaping genetic and phenotypic variation at the dispersal front. During range expansion, species advance through a series of founder events (i.e. bottlenecks), with major implications for genetic variation (Edmonds, Lillie & Cavalli-Sforza, 2004, Slatkin & Excoffier, 2012). These repeated founder effects in essence represent strong drift events, which can shape genetic variation at the dispersal front (Slatkin & Excoffier, 2012). It has for instance been shown that through the process called mutation surfing, new neutral mutations, or sometimes even disadvantageous mutations, can become dominant at the dispersal front through stochastic "sampling" of individuals during range expansion (Edmonds, Lillie & Cavalli-Sforza, 2004, Excoffier & Ray, 2008, Hallatschek & Nelson, 2008). As a consequence of these founder events, drift has been argued to play a major role during range expansion, by driving divergence at the dispersal front (Hallatschek, Hersen,

Ramanathan et al., 2007). This implies range expansion may have very significant implications on neutral evolution (McInerny, Turner, Wong *et al.*, 2009) or even non-neutral evolution through these strong stochastic drift effects acting on the population at the dispersal front.

Understanding evolutionary and ecological responses along during range expansion will thus be of vital importance to anticipate the survival or extinction of species due to climate change. In recent years, many field studies have been performed in order to observe eco-evolutionary responses along dispersal fronts (usually from invasion cases) (Chuang et al., 2016), as well as several modelling approaches (Alex Perkins, Phillips, Baskett et al., 2013; Phillips et al., 2010a; Travis et al., 2002). Unfortunately, field studies regularly suffer from the drawback that environmental conditions along the dispersal front are not constant which, in combination with the conflicting results concerning trade-offs, complicates interpretation. Therefore, in order to make predictions concerning adaptive responses along dispersal fronts, a thorough assessment of changes in selection pressures along dispersal fronts is necessary. Firstly, due to regular colonisation of new habitat, individuals at the edge will experience continuously lower densities compared to individuals at the core (Phillips et al., 2010b; Travis et al., 2002). Secondly, core and edge demes may differ in terms of environmental conditions, resulting in different selection pressures along the dispersal front (Chuang et al., 2016). Adaptation towards tolerance for lower temperatures was recorded for the brown anole (Anolis sagrei) (Kolbe, Ehrenberger, Moniz et al., 2014) and the cane toad (Rhinella marina) (Urban, Phillips, Skelly et al., 2007), two species that recently expanded their range. Changes in development time in the two-spotted spider mite have been shown to be linked to environmental variation along the dispersal front (Van Petegem et al., 2015). Corticosterone responses were found to be higher for house sparrows (Passer domesticus) at the edge of the a dispersal front, which may aid in coping with new environments or impressions (new stressors) (Liebl & Martin, 2012). Thirdly, conditions along the dispersal front may induce phenotypic variation through plasticity induced by experienced conditions, contrary to the first and second type of response, where there is a genetic basis. Especially density conditions can significantly affect dispersal behaviour through these kinds of interactions, as a mechanism to avoid for instance kin competition and inbreeding. (Benard & McCauley, 2008; Bonte et al., 2012; Bowler et al., 2005) These density effects are expected to mainly influence the motivation to disperse (Benard et al., 2008; De Meester & Bonte, 2010), whereas the driving force behind dispersal ability is associated with spatial sorting and assortative mating. Density effects and maternal effects have already been shown to influence evolution of dispersal traits in the two-spotted spider mite (Bitume, Bonte, Magalhaes et al., 2011), and model approaches predict evolution of phenotypically regulated dispersal traits for seed dispersal of pants (Ronce, Brachet, Olivieri et al., 2005). Whereas the first kind (spatial selection during range expansion) and third kind

(plasticity induced by experienced conditions) of adaptive responses arise due to intrinsic characteristics of the dispersal front (i.e. spatial distribution of individuals, density gradient and relatedness), the second category is dependent on environmental conditions associated with specific geographical locations. Such adaptations linked to environmental variation may differ between dispersal fronts, complicating interpretation of eco-evolutionary responses (Chuang et al., 2016). As a result of the combination of spatial sorting and density gradient shaping dispersal fronts, both genetic (evolutionary) adaptation as well as phenotypic plasticity (ecological adaptation) are expected to be of major importance for species persistence during periods of climate change (Reed, Schindler & Waples, 2011).

Objectives

By applying an experimental approach to study range expansion, we aim to 1) avoid confounding environmental variation and 2) separate the ecological responses to density effects from evolutionary adaptations, allowing for a thorough and systematic study of eco-evolutionary responses associated with range expansion. To our knowledge, there have been few attempts to clearly separate ecological and evolutionary responses along dispersal fronts by applying a systematic experimental approach. Therefore we designed two experiments consisting of replicated artificial metapopulations in order to simulate expanding dispersal fronts, using the two spotted spider-mite (*Tetranychus urtica*e Koch) as a study system. By working under controlled laboratory conditions we can avoid confounding responses linked to environmental variation (e.g. differences in temperature, light conditions, host plants, etc.), a factor which often complicates field studies. By limiting evolutionary adaptation in some of the metapopulations of both experiments, we aim to systematically disentangle ecological and evolutionary responses. This will allow us to distinguish between evolutionary adaptation (i.e. spatial selection) directly related to dispersal fronts and their typical spatial distribution of individuals (expected to be mainly adaptations towards increased dispersal and fast reproduction) and ecological responses acting along dispersal fronts (for instance phenotypic plasticity induced by different density conditions). In order to achieve this goal, we will test a broad suite of traits linked to dispersal behaviour, morphology, functional response (i.e. food ingestion) and life history strategy. Furthermore, by comparison with field data collected and analysed earlier by Van Petegem et al. (Van Petegem et al., 2015), we will be able to better comprehend and distinguish responses linked to range shifts and responses related to environmental variation. These new insights will contribute to a better understanding and prediction of eco-evolutionary responses along dispersal fronts (be it due to climate induced range shifts or invasions), which will be vital to anticipate future impacts on distribution and

persistence of species, in the context of a world increasingly affected by anthropogenic influences.

Material and methods

Study system

The two-spotted spider mite (Tetranuchus urticae Koch – Acari, Tetranichidae) is a haplodiploid phytophagous mite species, with a global distribution. The species can achieve densities up to 50 individuals/cm² (Helle & Sabelis, 1985), and can consequently cause severe damage to crops. Development happens through a series of mobile and immobile stages, with transition from immobile to mobile stage through ecdysis of the mite. This allows for clear distinction between juveniles (with the teleiochrysalis stage the last immobile stage prior to becoming adult) and adults. Sex-ratio is typically female biased (Krainacker & Carey, 1989) but can be influenced by the mother (Young, Wrensch & Kongchuensin, 1986). Several genetic strains were used during the experiments. The LS-VL strain was collected from rose plants in Ghent in the year 2000 (Van Leeuwen, Tirry & Nauen, 2006). Five inbred strains (MR-VP, SR-VP, JPS, ALBINO and LONDON) were obtained courtesy of the research group of Thomas van Leeuwen. Inbred (isofemale) strains were created by means of repeatedly crossing female mites with her male offspring, for several generations until genetic variation had been almost completely reduced. After collection, stock populations were maintained on common bean (Phaseolus vulgaris variety Prelude) in a climate controlled room at 26°C ± 0,5°C, 60% RH and 16/8 h (L/D) photoperiod. From the five inbred strains, a mixed strain was created by means of reciprocal crossings. Females of each line were crossed with males from all other lines, and the female offspring (F1 offspring) of the inseminated females were collected and allowed to oviposit (resulting in F2 offspring). Subsequently, we performed further crossings starting with the F2 offspring, following the same protocol, and a third crossing with the F2 offspring of the second crossing. Finally, the F2 offspring of the third crossing were placed together on bean plants, in order to maintain a stable stock population (from here on denoted as MIX). All reciprocal crossings were successful, resulting in a MIX stock population which should contain genetic material of all inbred lines. Common bean plants (Phaseolus vulgaris, variety Prelude) were used as host plant for all stock populations and in all experimental setups. Stock populations of the LS-VL and MIX strain were maintained on whole bean plants, and placed in plastic bins in a climate controlled room (28,1°C ± 2,1°C) with a 16/8h (L/D) photoperiod. The stock populations of the inbred strains were kept on bean leaf patches placed in moist cotton in a petri dish. The stock populations of inbred strains were kept in separate

incubators at 28°C with a 16/8h (L/D) photoperiod prior to start of the experimental setup, in order to avoid accidental crossbreeding between different strains.

Synchronisation

Prior to starting the experiments and trait assessments, we synchronised the mites as to exclude direct environment-induced maternal effects and to ensure range expansion experiments and the trait assessments were started with females of the same age. In order to synchronise mites, adult females were collected and placed on delineated leaf patches of 3,5cm by 4,5cm with a density of five mites per patch. Subsequently, these patches were placed under common garden conditions in an incubator at 30°C with a 16/8h (L/D) photoperiod. The mites were allowed to oviposit for 24 hours, after which the adult females were removed. The offspring was left to develop under common garden conditions in the incubators until 24 hours after the female offspring reached the adult stage. At this stage, adult females were collected for use in the experiments or trait assessments. As males usually guard females in the last moulting phase and mate with them shortly after the females reach the adult stage, collecting females approximately 24 hours after reaching the adult stage ensures collected females had already mated.

Range expansion experiments

Two separate experiments were designed in which expanding metapopulations were created using the same basic experimental setup. This setup consisted of plastic trays filled with moist cotton in which a series of 4cm² square leaf patches was placed. Leaf patches were delineated with paper strips (in order to reduce mite mortality by accidental drowning) and connected by means of parafilm bridges of 8cm by 1cm. These trays were placed in a climate controlled room with a temperature of 28,1°C ± 2,1°C and a 16/8h (L/D) photoperiod. Initially, four connected patches were placed in each tray and ten synchronised females were placed on the first patch of every tray. For both experiments, twelve of such metapopulations were created. Thrice every week the densities of female adults were counted on all patches and in case adult female mites had expanded their range to the last or next to last patch, the dispersal front was extended as to always maintain two non-colonised patches at the edge of the dispersal front. In order to ensure continuous survival of mites on the setup, leaf patches were renewed weekly. Renewal of leaf patches was done by moving all one week old patches aside (replacing two week old patches that were placed there a week earlier) and placing new patches in their stead. This allowed mites to walk from the old patches onto the new ones, thus maintaining each subpopulation along the dispersal front. In both experiments, evolutionary responses were constrained in half of the metapopulations, thus only allowing for ecological responses (i.e. transgenerational plasticity linked to for instance the density gradient and kin structure

along the dispersal front). Evolutionary potential was not constrained in the remainder of the setups, allowing for both evolutionary and ecological responses to act along the dispersal front. This setup was maintained eighty days (approximately ten generation times). After the allotted period for the range expansion experiment had passed, individuals were collected from the start patches (core) and end patches (edge) of the setup for assessment of life history and dispersal associated traits.

Experiment 1

In the first experiment, mites of the genetically diverse LS-VL strain were used. After synchronisation, ten adult females were placed on the first patch of each of the twelve metapopulations. Six of the metapopulations were not manipulated, allowing for both evolutionary responses as well as ecological responses (transgenerational plasticity) to act. The non-manipulated metapopulations of experiment will henceforth be referred to as ECO-EVO1. In the other six metapopulations, all adult females were removed weekly and replaced by the same number of adult females, collected from the stock population. The metapopulations where replacements were performed will as of now be referred to as RFS[1]. Replacement individuals were selected based on age of the original individuals, as to avoid impacting demographic features of subpopulations. These replacements were done every week for the duration of the range expansion experiment, up until two weeks prior to collection of individuals for analysis. By exchanging adult females with stock individuals on a weekly basis, metapopulations were influenced in two ways. Evolutionary responses through spatial selection were prevented as all individuals were exchanged with a random subset of the stock population, and kin structure was effectively destroyed, due to prevention of high relatedness at the dispersal front. A similar method has already been proven to effectively prevent evolution in a microbial system (Livingston, Matias,

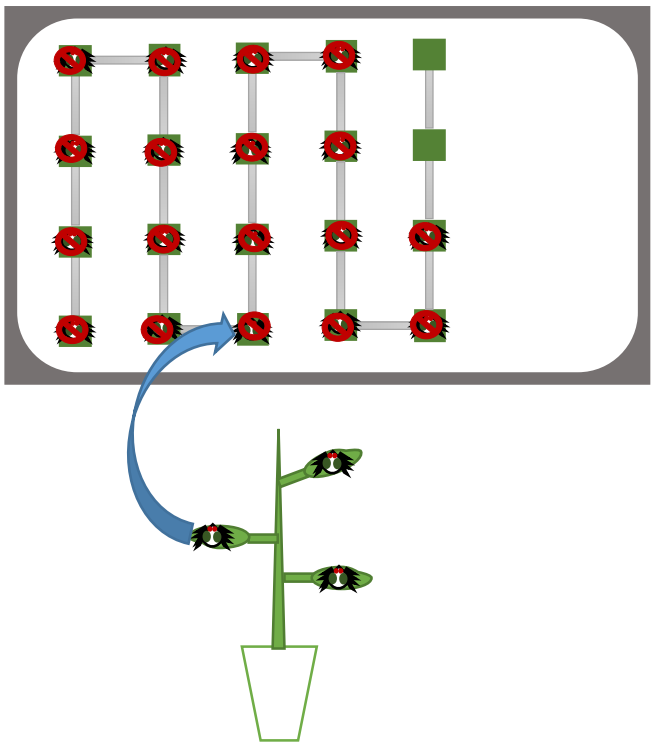

Fig. 1: Depiction of one metapopulation (replica) used in experiment 1. All adult females of the RFS treatment were replaced every week with individuals of the stock population

[1] Replacement From Stock

Calcagno et al., 2012). The use of this method allowed to assess the combined effects of both spatial selection and increased relatedness (and consequential kin competition) at the dispersal front.

Experiment 2

Just like for the first experiment, twelve expanding metapopulations were created for the second range expansion experiment. In six of those, evolutionary responses were again constrained, but by using a different approach compared to the first experiment. In the remaining six metapopulations, no such constraints of evolutionary responses were enforced. Evolutionary responses were constrained by the use of inbred mite lines. Inbred lines contained hardly any genetic variation, constraining evolutionary responses through selection, as standing genetic variation needed for such adaptive responses is simply lacking. Three of the aforementioned inbred lines (LONDON, SR-VP and JPS)[2] were used for the creation of six metapopulations. For each inbred line, two replicates (i.e. metapopulations) were created for the range expansion experiment, on which 10 synchronised adult females of that specific line were placed at the start of the experiment. This allowed to have three true replicas (the three inbred strains) and two pseudo-replicas per line (thus for a total of six evolutionary constrained metapopulations). These metapopulations using inbred lines are as of now referred to as ECO. Prior to the start of the range expansion experiment, DNA of the inbred lines was sequenced and checked for the occurrence of double peaks at specific locations (which would be indicative for crossbreeding between different lines), in order to ensure inbred lines were still inbred at the start of the experiment. Although spatial selection is inhibited by the use of the inbred lines, transgenerational plasticity associated with density conditions and relatedness can however still occur. Whilst this setup does not constrain the possibility for adaptive responses through de novo mutations, this is unlikely to be an important factor due to the relatively short time the selection experiment was maintained. The use of inbred lines has already been demonstrated to effectively constrain evolutionary responses in an aphid system (Turcotte, Reznick & Hare, 2011). The remaining six metapopulations (henceforth referred to as ECO-EVO2) were created using the MIX line. The MIX line contained at least a minimum of standing genetic variation, through the crossbreeding of all different inbred lines. As such, both evolutionary adaptation through spatial selection and transgenerational plasticity effect associated with population structure (density and relatedness) can act in setups using the MIX line. The metapopulations using the MIX line are henceforth referred to as ECO-EVO2. Whereas in the

[2] We intended to create two replicas for all five lines, however the ALBINO stock population went extinct for unknown reasons, and the MR-VP line reproduced too slow to survive the experimental setup, resulting in extinction on all patches early on in the experiment. For these reasons we had to omit the MR-VP and ALBINO line from the experiment.

first experiment the combined effect of kin structure as well as spatial selection was prevented, only spatial selection is prevented in the second experiment. The combination of both experiments thus allowed us to assess the effects of spatial selection, density gradient and relatedness separately.

Trait assessments and statistical analysis

In order to assess life history and dispersal traits, mites were collected from the stock populations of all strains prior to the range expansion experiment, and from the core and edge subpopulations of all replicas (i.e. from every metapopulation) of both range expansion experiments, after ten generations. Henceforth for the trait assessments and all statistical analyses, the term “Treatment” will be used to denote whether individuals come from the stock population (Stock), or from either the evolutionary constrained (RFS for experiment 1, ECO for experiment 2) or unconstrained (ECO-EVO1 for experiment 1, ECO-EVO2 for experiment 2) metapopulations. The term “Patch” indicates whether individuals within a treatment come from the core of the metapopulation (Start) the edge (End) or from the stock (before). After mites were collected from the stock populations and core and edge subpopulations of every metapopulation they were synchronised, in order to use their offspring for the trait assessments. Four different setups were created in order to test all traits (discussed in detail below). Every trait assessment setup was created for all collected and synchronised mites (stock individuals and core and edge individuals of every replica). The assessed traits were compared between the core and dispersal front populations, in order to determine whether range expansion led to either evolutionary responses or transgenerational plasticity. Furthermore, both core and dispersal front populations were compared with the original stock population, however the results of these analysis are discussed in the appendix, as the focus on this work is in trying to detect eco-evolutionary responses, rather than determining whether they occur at the core population or at the dispersal front. All statistical analyses for trait assessments were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

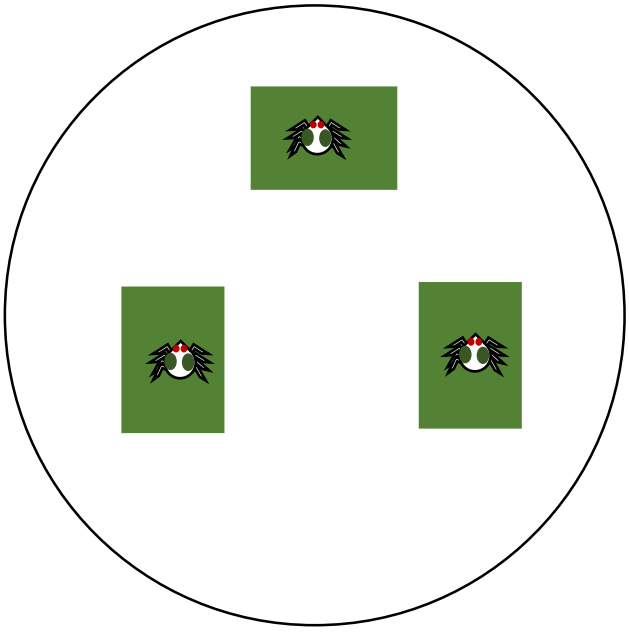

Fig. 2: Petri dishes used for the EJDSA and FLL trait assessments.

The first setup for trait assessments (henceforth referred to as EJDSA[3]) consisted of petri dishes with moist cotton, in which three delineated bean leaf patches of 1,5cm X 2,5cm were placed. On each leaf patch, a single synchronised adult female was placed. Subsequently, dishes were placed in an incubator at 20°C with a 16/8h (L/D) photoperiod. Females were allowed to oviposit for 24 hours, and were subsequently removed from the patch. If after 24 hours, no (or too few) eggs had been laid, the eggs were removed and the females were allowed to oviposit for another 24 hours, as to ensure sample sizes were large enough to be statistically relevant. After removal of females, all patches were checked every 24 hours to assess the number of living offspring and their developmental stage[4] at that time, until all individuals had reached the adult life stage. Female mites were removed from the leaf patch immediately after reaching the adult stage, and placed on a new patch, on which they were left to feed for two days. Subsequently the females (who at that time had reached the adult life stage for 48 hours to 72 hours) were placed in an Eppendorf tube and frozen at a temperature of -80°C. After all females were collected from the EJDS setup and had been frozen for at least a several hours, they were removed from the freezer and placed on a cover glass with length markings. Subsequently, a Nikon Coolpix 4500 binocular-mounted camera was used to photograph the females for morphometric analysis. The outline of the body was traced (excluding legs and gnathosoma) in ImageJ and used to calculate surface area of the body, perimeter of the outline and the Feret's diameter. The markings on the measuring plate were used to standardise measurements across all photographs. Survival statistics (juvenile survival and egg survival), sex ratio and development time were inferred from the EJDSA setup. Egg survival was calculated as the proportion of eggs of a single female developing into larvae, juvenile survival as the proportion of larvae of one female reaching the adult stage. Sex ratio was calculated as the proportion of male offspring to all offspring of one female. Development time was analysed separately for female and male individuals, as responses may differ between the sexes, and was counted as the number of day between

[3] Egg survival, Juvenile survival, Development time, Sex ratio and Adult size
[4] See section study system

laying of the egg and reaching the adult stage. Only surface area was included in the analyses as measure of adult size. Egg survival, juvenile survival and sex ratio were assessed using generalized linear mixed models (following a Bernoulli distribution for egg and juvenile survival and a binomial distribution for sex ratio), with patch as fixed effect and petri dish, replica, the interaction replica with patch (only for comparison of start and end patches within a treatment[5]) and line (for the second experiment) included as random effects. Adult size was tested using a mixed model with patch as a fixed effect and replica, the patch-replica interaction, line and person analysing the pictures[6] as random effects. Development time was analysed separately for male and female individuals, using mixed models. Patch was included as a fixed effect, and leaf in the petri dish (i.e. mother of the individual), petri dish, replica, replica-patch interaction and line were included as random effects.

For the second setup (from here on referred to as FLL[7]), petri dishes were prepared in the same way as for the EJDSA setup (with 3 leaf patches per petri dish and one synchronised female per patch), but placed in an incubator at 30°C with a 16/8h (L/D) photoperiod. Prior to placing females on each patch, all patches were photographed using a Nikon D3200 mounted camera with sidewise led lighting, oriented with an angle of 45° towards the photographed surface through a blue coloured light filter. After females were introduced on leaf patches, all eggs laid by the female were counted and removed daily until death of the female. After three and five days, all patches were again photographed. These pictures were later analysed in ImageJ in order to determine leaf consumption by mites (consumed cells were drained of chlorophyll and consequentially had a typical white colour, allowing for clear distinction between consumed and healthy cells). Lifetime fecundity was assessed as the sum of all offspring produced during the entire lifespan of the mites and longevity as the number of days until death after being placed on the leaf patch. Mean daily fecundity was calculated as the mean number of eggs produced by female mites each day. Data points for daily mean fecundity were only included if females spent the entire day uninterrupted on the patch (day of death or interrupted days[8] were omitted). Cumulative fecundity was assessed as the cumulative number of offspring with each day. As fecundity typically decreased with increasing age, time (days) was log transformed in order linearize the relation between cumulative fecundity (dependent

[5] In all analyses, the interaction of replica with patch was included whenever start and end patches were compared within a treatment, in order to account for differences in response strength between replicas. When start and stock or end and stock patches were compared, this interaction was not necessary, as in that case replica and replica*patch would statistically imply the same as including replica nested within treatment, which was already included in the model.

[6] Multiple people analysed the pictures, and thus this was included in the random effects in order to account for possible measurement bias.

[7] Fecundity, longevity and leaf consumption

[8] Female mites sometimes accidentally walked in the moist cotton, in which they remained stuck until being manually placed back on the patch the next day.

variable) and time (independent variable). All five traits (longevity, lifetime fecundity, mean daily fecundity, cumulative fecundity and leaf consumption) were assessed using mixed models. Patch, log(day) and the interaction patch with log(day) were included as fixed effects for cumulative fecundity. For all other traits, only patch was included as fixed effect, and petri dish, replica, replica-patch interaction and line were included as random effects. For mean daily fecundity and cumulative fecundity, individual laying the eggs was included as repeated measure.

The third setup (henceforth referred to as POPGR[9]) consisted of petri dishes with a single 5,5cm by 5,5cm delineated bean leaf patch placed in moist cotton. The petri dishes were placed in an incubator at 30°C with a 16/8h (L/D) photoperiod. A single adult female was placed on the patch in order to oviposit. Starting from the eighth day after placing the adult female on the leaf patch, the number of female adults on the patch was counted weekly (for the first experiment) or twice weekly (for the second experiment). Population growth rate was inferred from the POPGR setup. Data points were omitted from the analysis if 1) the initial adult female placed on the patch was not inseminated[10], 2) if the population size started to decrease strongly due to complete exploitation of the leaf or by consumption or 3) if the leaf patch started to decompose (not because of leaf consumption but due to old age or rot), strongly affecting performance of mites. The data was transformed to the natural logarithm of the number of adult females, as the number of adult females increased exponentially with time. Population growth rate was assessed using a linear mixed model with patch, day and the interaction patch*day as fixed effects, and replica, the replica*patch interaction and line as random effects. Petri dish was included as repeated measure.

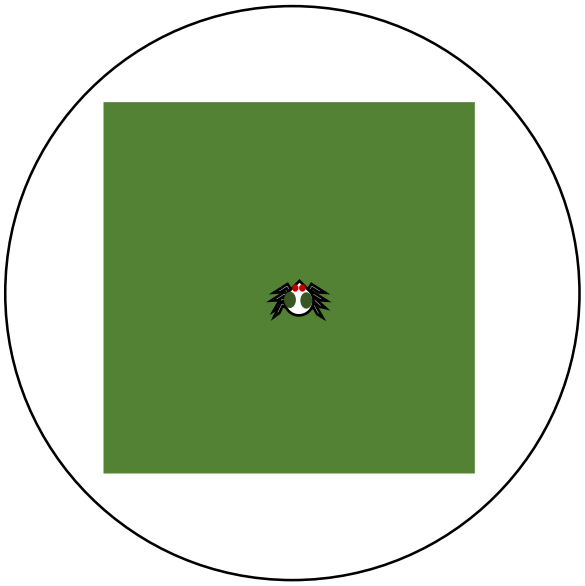

Fig. 3: Petri dishes used for the POPGR trait assessments

[9] Population Growth

[10] Due to the haplodiploid reproductive system, non-inseminated females produce only male offspring, allowing for clear distinction between inseminated and non-inseminated females.

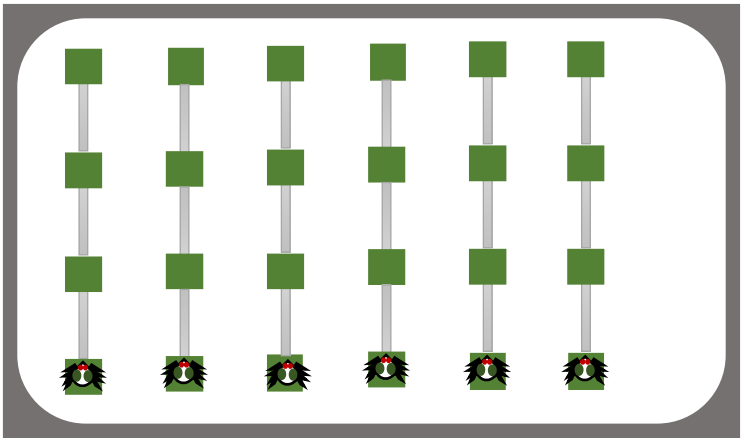

Fig. 4: Setup used for the AMB DISP trait assessment, consisting of several transects of four connected bean leaf patches with 10 synchronised adult females placed on the first patch.

For the last setup (as of now referred to as AMB DISP[11]), a series of four delineated bean leaf patches of 2cm by 2cm, connected by 8cm parafilm bridges was created. Ten adult females were placed on the first patch. This setup was placed in a climate controlled room at a temperature of 28,1°C ± 2,1°C at a 16/8h (L/D) photoperiod. During a week, females on all patches and bridges were counted in order to determine emigration and long distance dispersal rates. Mean distance moved was assessed by summing for all living mites the distance from the start patch (e.g. distance is 2 if mite is on patch 3), and dividing this sum by the total number of living mites. Mean distance moved was assessed using a linear mixed model, with patch as fixed effect, and line and replica[12] as random effects. Distance moved was assessed for the fourth day, as trends were clearest on that day.

Apart for the trait assessments themselves, the rate of advance of the dispersal front was compared between the evolutionary constrained and unconstrained replicas during the range expansion experiments. A mixed model with Treatment, time (days since the start of the range expansion experiment) and their interaction as fixed effects was used. Replica of the range expansion experiment was included as repeated measure, and line as random variable.

In case the first analysis did not yield any clear indications that a particular trait was under selection, and graphs (see appendix) suggested there may be drift effects acting on assessed traits, a second analysis was performed in which the contribution of such possible drift effects was tested. These analyses of drift effect tested separately for all treatments (ECO-EVO1 treatment and RFS treatment of experiment 1 and ECO-EVO2 and ECO treatment of experiment 2) whether there were significant drift effects. The occurrence of two types of drift components was tested. Firstly, differences between metapopulations (the replica variable) were tested, in order to detect the effect of initial population (i.e. whether initial population at the beginning of the range expansion experiment differed between metapopulations) and the

[11] Ambulatory Dispersal

[12] Only for experiment 2, as there was only on ambulatory dispersal transect per replica for the first experiment.

occurrence of strong drift effects at the start of the range expansion experiment.[13] Secondly, differences in response within metapopulations (the interaction between the replica and patch parameters) were tested, in order to assess whether stochastic divergence between the low density conditions at the dispersal front led to stochastic divergence between replicas. A total of eight traits (development time, sex ratio, adult size, lifetime and mean daily fecundity, longevity, leaf consumption and ambulatory dispersal) were finally assessed for the occurrence of aforementioned drift effects, after consideration of trait assessments and interpretation of graphs. Statistical models for drift assessment were similar as those above for each trait, however for drift assessment, both replica, patch and their interaction were included as fixed effects for both treatments of the first experiment and the ECO-EVO2 treatment of experiment 2, and line, patch, replica, and the interaction between these three for the ECO treatment of the second experiment. Consequentially, replica, line and the replica-patch interaction were removed from the random effects in these models. Statistical output of trait assessments and drift assessments is included in the form of tables under every section. Additional graphs and statistical output can be found in the appendix.

In order to assess whether there was covariance of traits within start and end patches of different replicas, a multivariate analysis was performed. Expected values for all traits were determined for the start and end patch of every replica for all treatments, using the statistical models in SAS. Subsequently, this data was analysed separately for both experiments in Canoco 5 using an unconstrained principal components analysis.

Results

Range expansion

Rate of dispersal front advance

The position of the dispersal front increased significantly faster for the ECO-EVO1 treatment in the first range expansion experiment, compared to the RFS treatment (Fig. 5, **$F_{1,413}$=101.46; p<0.0001**). Differences between the two treatments arise early on in the experiment (after approximately 20 days). The difference at the end of the range expansion experiment is quite extensive, with mean length of the metapopulation approximately 17 patches for the RFS treatment, about 23 patches for the ECO-EVO1 treatment. The same trend was found for the second experiment, with advance of the dispersal front significantly higher for the ECO-EVO2

[13] Whether differences between metapopulations were the result of these initial differences in genetic composition of subpopulations, or they occurred to strong initial drift effects on these subpopulations could not be discerned from each other.

treatment compared to the ECO treatment (Fig. 6, **$F_{1,399}$=7.03; p=0.0083**). For the second experiment however, even though the difference was significant, divergence between evolutionary constrained and unconstrained treatment arises only late during the experiment (later than 40 days), and both treatments end up with a mean maximal length of over 20 patches at the end of the range expansion experiment (approximately 21 for the ECO treatment and 22 for the ECO-EVO2 treatment).

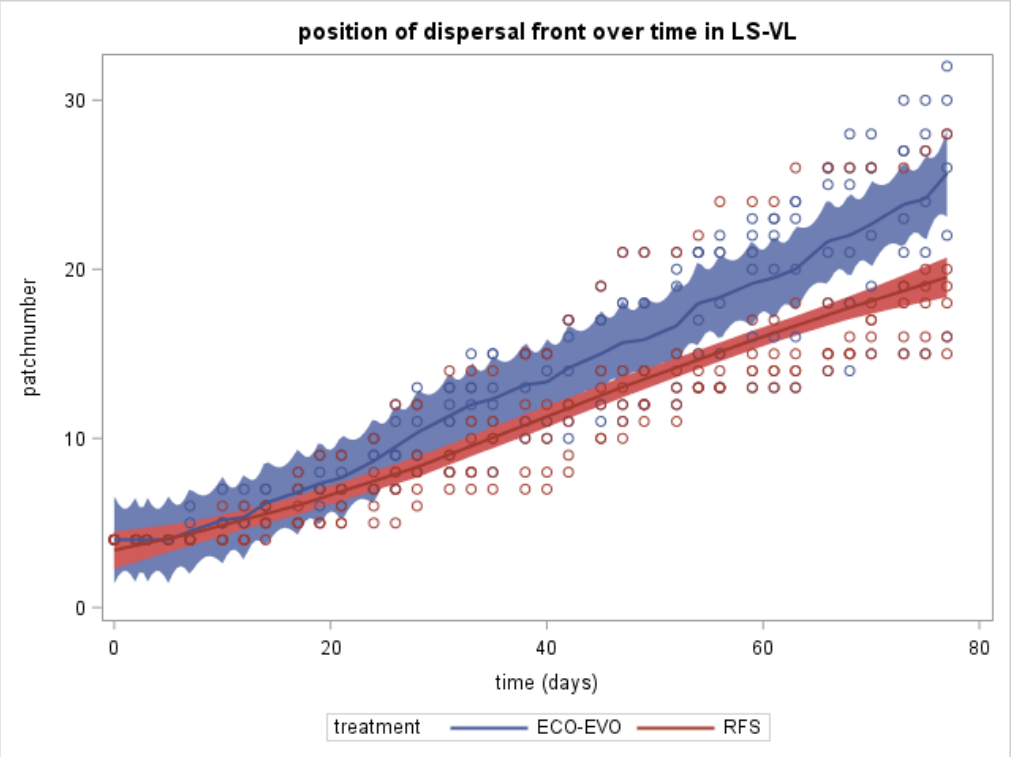

Fig. 5: Length of metapopulation over the duration of the first range expansion experiment, for the ECO-EVO1 and RFS treatments

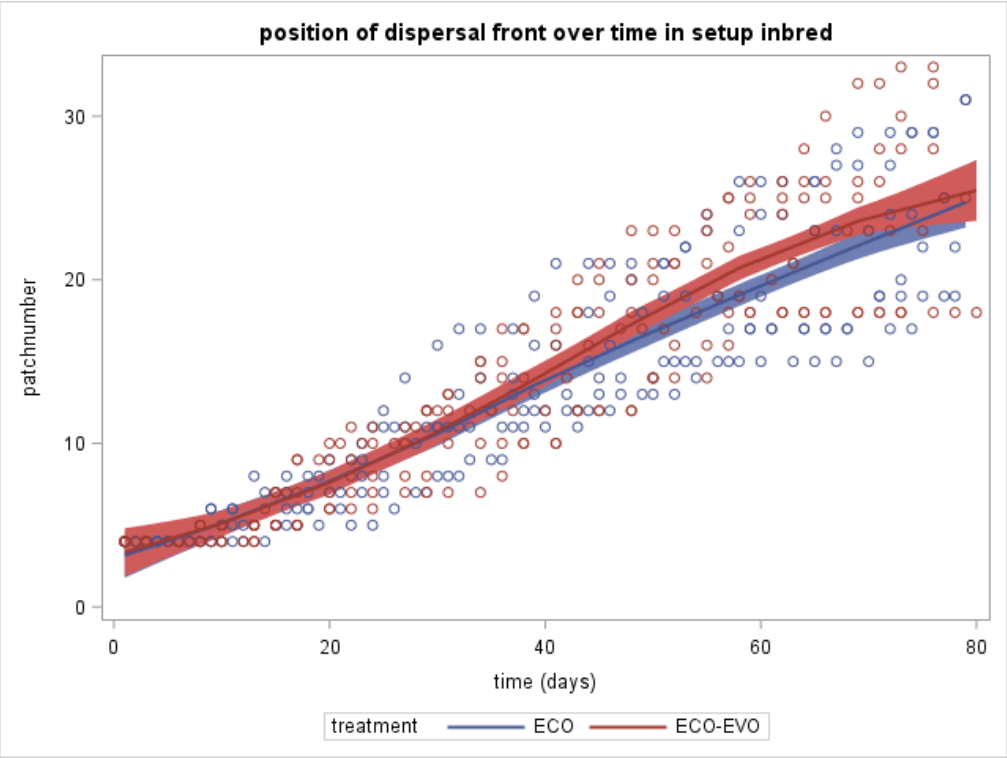

Fig. 6: Length of metapopulation over the duration of the first range expansion experiment, for the ECO-EVO2 treatment (MIX line) and ECO treatment (JPS, LONDON and SR-VP lines).

Life history trait assessments

Population growth rate

In the evolutionary unconstrained setups, population growth rate was significantly higher at the dispersal front compared to the core population, both for the first experiment (fig. 7, **$F_{1,153}$=5.32; p=0.0225**) and for the second experiment (fig. 9, **$F_{1,235}$=6.46; p=0.0117**). Population growth rates did however not differ between the dispersal front and the core population for either the first (fig. 8) or the second (fig. 10) experiment. The increase in population growth rate could have arisen through adaptation of many life history traits., which are discussed in the next sections.

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	Slope: **$F_{1,153}$=5.32; p=0.0225** Intercept: $F_{1,8.64}$=0.00; p=0.9823	Slope: $F_{1,117}$=0.31; p=0.5817 Intercept: $F_{1,10.3}$=0.69; p=0.4261
Experiment 2 ECO-EVO2-ECO	Slope: **$F_{1,235}$=6.46; p=0.0117** Intercept: $F_{1,9.45}$=1.12; p=0.3159	Slope: $F_{1,278}$=0.51; p=0.4747 Intercept: $F_{1,20}$=0.25; p=0.6256

Table 1: Statistical output for comparison of populations from the core and dispersal front of both experiments, for population growth rate

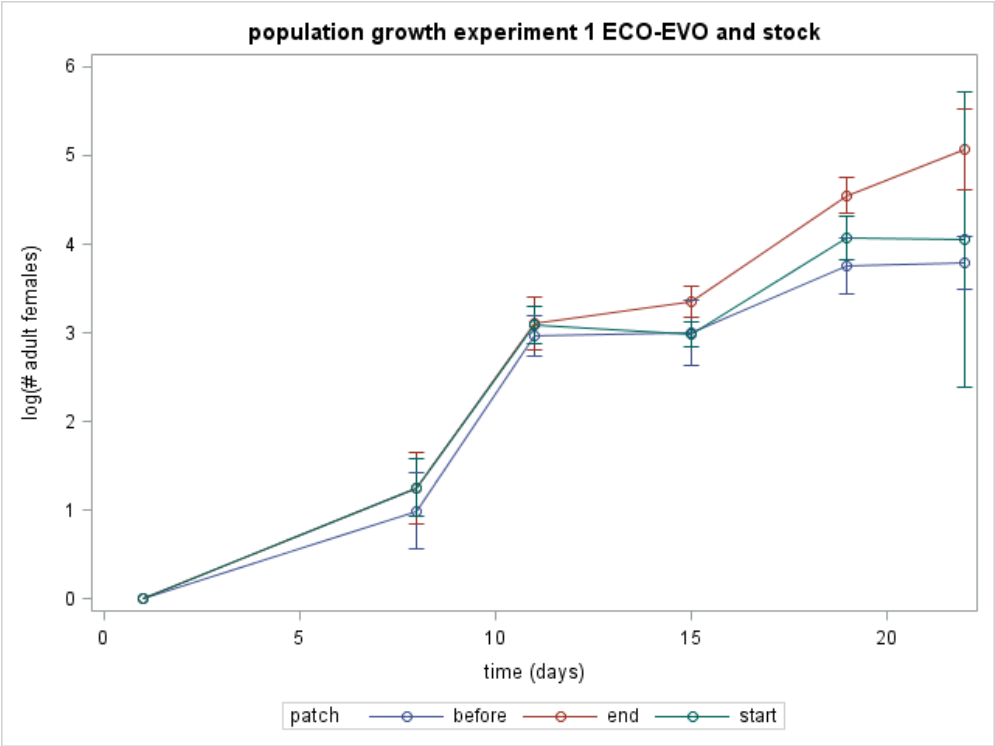

Fig. 7: Population growth rate by patch for the ECO-EVO1 and stock treatments of experiment 1. X-axis: time in days since adult female was placed on the patch. Y-axis: logarithm of the density of adult females. Flags represent confidence limits.

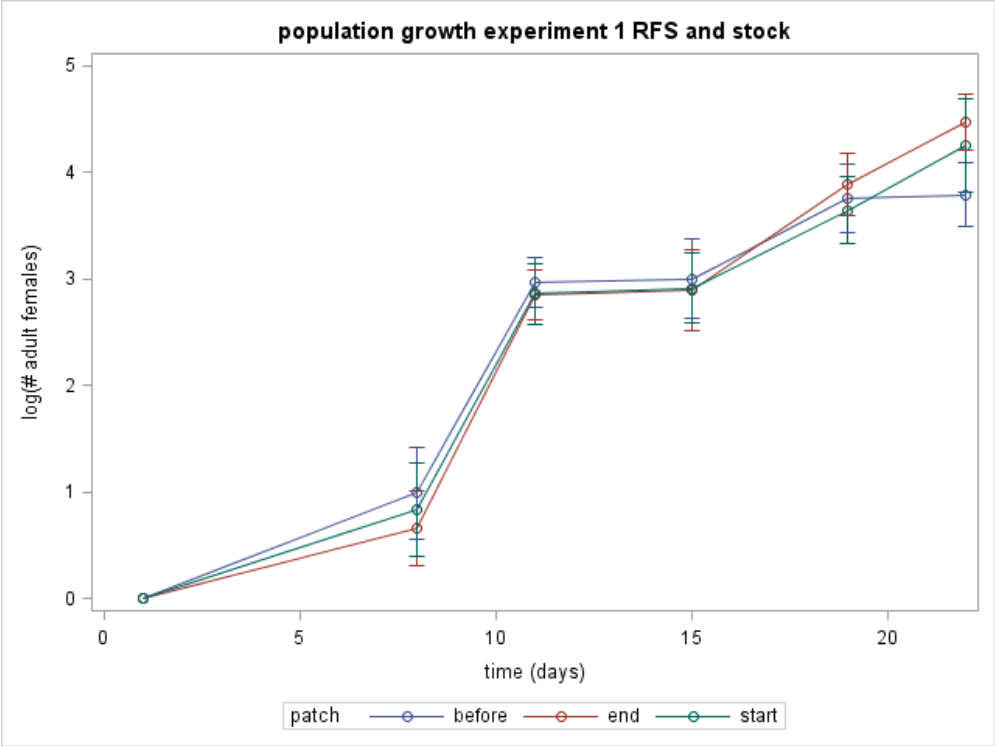

Fig. 8: Population growth rate by patch for the RFS and stock treatments of experiment 1. X-axis: time in days since adult female was placed on the patch. Y-axis: logarithm of the density of adult females. Flags represent confidence limits.

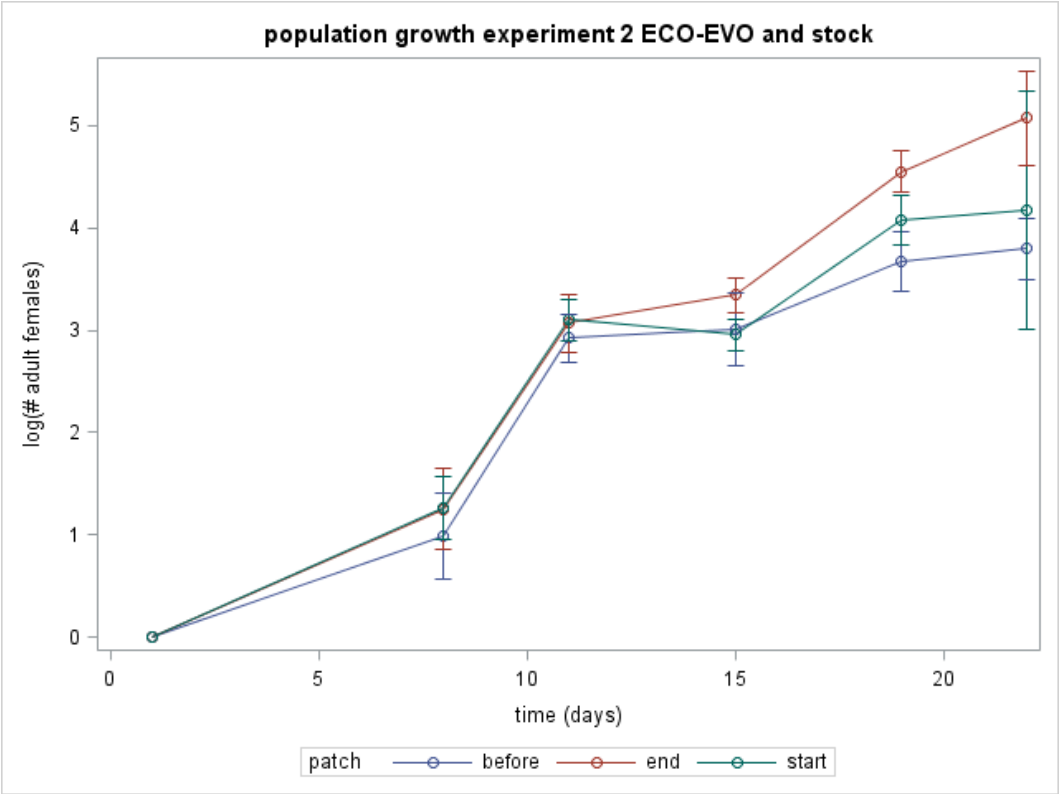

Fig. 9: Population growth rate by patch for the ECO-EVO2 and stock treatments of experiment 2. X-axis: time in days since adult female was placed on the patch. Y-axis: logarithm of the density of adult females. Flags represent confidence limits.

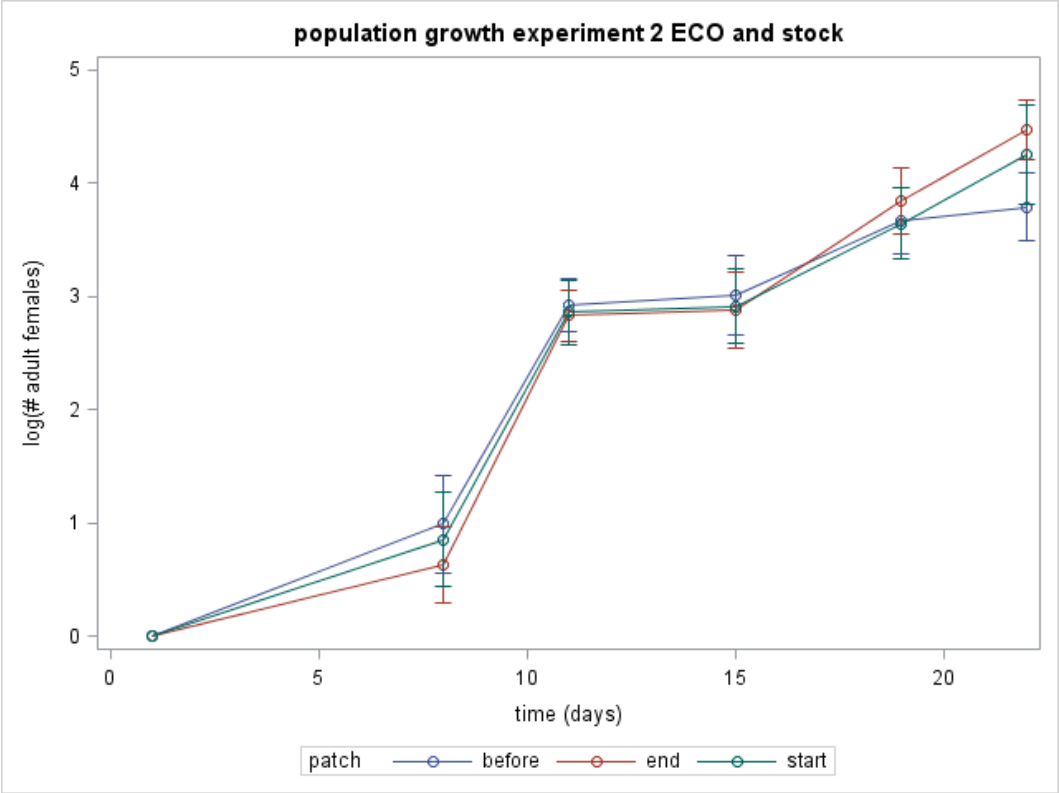

Fig. 10: Population growth rate by patch for the ECO and stock treatments of experiment 2. X-axis: time in days since adult female was placed on the patch. Y-axis: logarithm of the density of adult females. Flags represent confidence limits.

Egg survival

No differences in egg survival between core and dispersal front populations were found for the evolutionary unconstrained treatments of either experiment 1 ($F_{1,366.4}$=0.18; p=0.6718) or experiment 2 ($F_{1,6.685}$=0.74; p=0.4205). For the evolutionary constrained treatments, egg survival was (marginally) higher in case of the first experiment (**$F_{1,6.486}$=5.84; p=0.0490**), but this was not the case for the second experiment ($F_{1,8.774}$=0.17; p=0.6869).

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,366.4}$=0.18; p=0.6718	**$F_{1,6.486}$=5.84; p=0.0490**
Experiment 2 ECO-EVO2-ECO	$F_{1,6.685}$=0.74; p=0.4205	$F_{1,8.774}$=0.17; p=0.6869

Table 2: Statistical output for comparison of populations from the core and dispersal front of both experiments, for egg survival

Juvenile survival

In the evolutionary unconstrained treatments, no differences in juvenile survival could be detected for either experiment 1 (F1,1.746=0.10; p=0.7826) or the second experiment $F_{1,476.9}$=2.39; p=0.1226) . For the evolutionary constrained setups, juvenile survival only differed significantly for the second experiment (**$F_{1,5.284}$=10.77; p=0.0202**) but not for the first experiment ($F_{1,4.246}$=0.02; p=0.9020).

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,1.746}$=0.10; p=0.7826	$F_{1,4.246}$=0.02; p=0.9020
Experiment 2 ECO-EVO2-ECO	$F_{1,476.9}$=2.39; p=0.1226	**$F_{1,5.284}$=10.77; p=0.0202**

Table 3: Statistical output for comparison of populations from the core and dispersal front of both experiments, for juvenile survival

Development time

No differences in either female or male development time could be detected in either of both experiments. Note however that (especially for the first experiment) quite some variability can be seen between replicas (Fig. 11-14), suggesting drift effects may significantly affect development time.

Core vs. dispersal front female	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,2.25}$=0.66; p=0.4937	$F_{1,2.37}$=0.06; p=0.8328
Experiment 2 ECO-EVO2-ECO	$F_{1,7.69}$=0.02; p=0.8981	$F_{1,5}$=0.96; p=0.3719

Table 4: Statistical output for comparison of populations from the core and dispersal front of both experiments, for female development time

Core vs. dispersal front male	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,3.72}$=0.96 p=0.3860	$F_{1,142}$=0.09; p=0.7611
Experiment 2 ECO-EVO2-ECO	$F_{1,9.64}$=0.18; p=0.6845	$F_{1,6.28}$=0.11; p=0.7536

Table 5: Statistical output for comparison of populations from the core and dispersal front of both experiments, for male development time

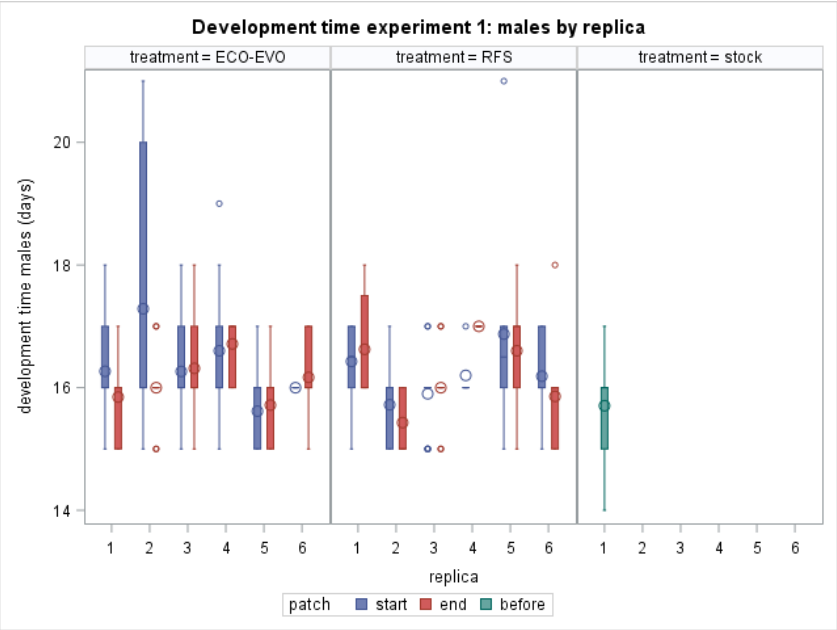

Fig. 11: Boxplots of male development time by replica and patch for experiment 1.

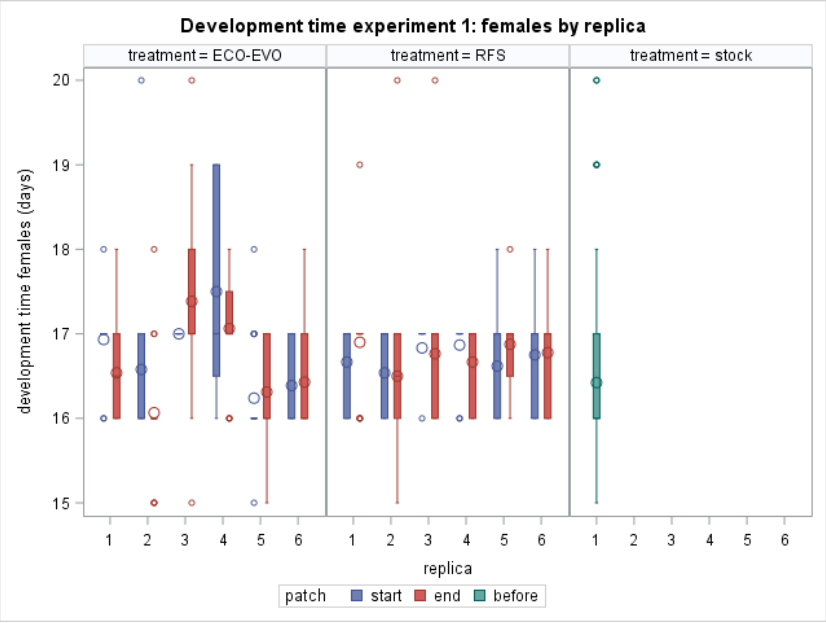

Fig. 12: Box plots of female development time by replica and patch for experiment 1

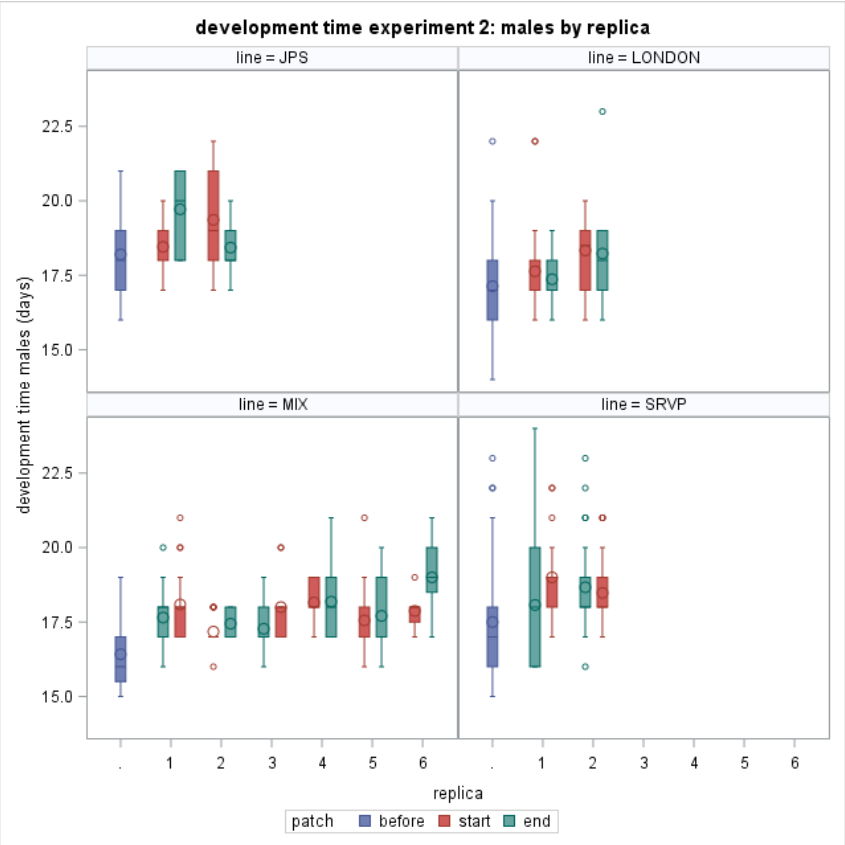

Fig. 13:Boxplots of male development time by replica and patch for experiment 2

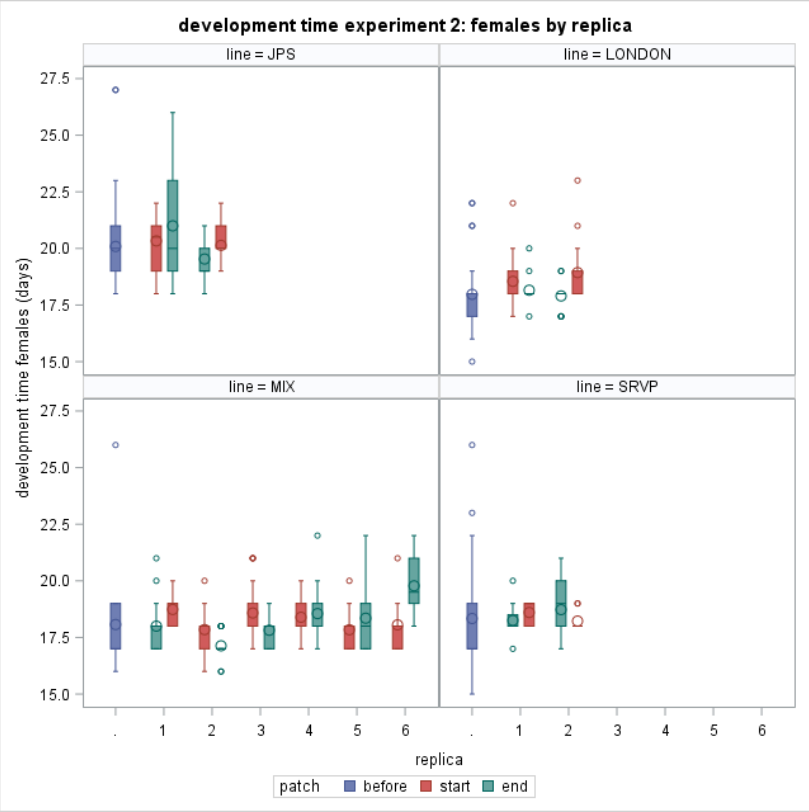

Fig. 14: Boxplots of female development time by replica and patch

Sex ratio

No significant differences in sex ratio between core populations and dispersal front could be detected for either the first or the second experiment.

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,3.612}$=1.25; p=0.3332	$F_{1,3.362}$=0.46; p=0.5402
Experiment 2 ECO-EVO2-ECO	$F_{1,129.6}$=0.71; p=0.4018	$F_{1,5.734}$=0.98; p=0.3622

Table 6: Statistical output for comparison of populations from the core and dispersal front of both experiments, for sex ratio

Adult size

Adult size did not differ between core and dispersal front populations for the evolutionary unconstrained (ECO-EVO1) treatment of the first experiment ($F_{1,3.48}$=0.52; p=0.5158). In the evolutionary constrained treatment (RFS) however, individuals from the dispersal front were significantly larger (**$F_{1,131}$=6.07; p=0.0151**) compared to individuals from the stock. In the second experiment, adult sizes did not differ between the core and dispersal front for either the evolutionary unconstrained (ECO-EVO2) treatment ($F_{1,4.87}$=1.28; p=0.3104) or the unconstrained (ECO) treatment ($F_{1,4.66}$=0.52; p=0.5055).

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,3.48}$=0.52; p=0.5158	**$F_{1,131}$=6.07; p=0.0151**
Experiment 2 ECO-EVO2-ECO	$F_{1,4.87}$=1.28; p=0.3104	$F_{1,4.66}$=0.52; p=0.5055

Table 7: Statistical output for comparison of populations from the core and dispersal front of both experiments, for adult size

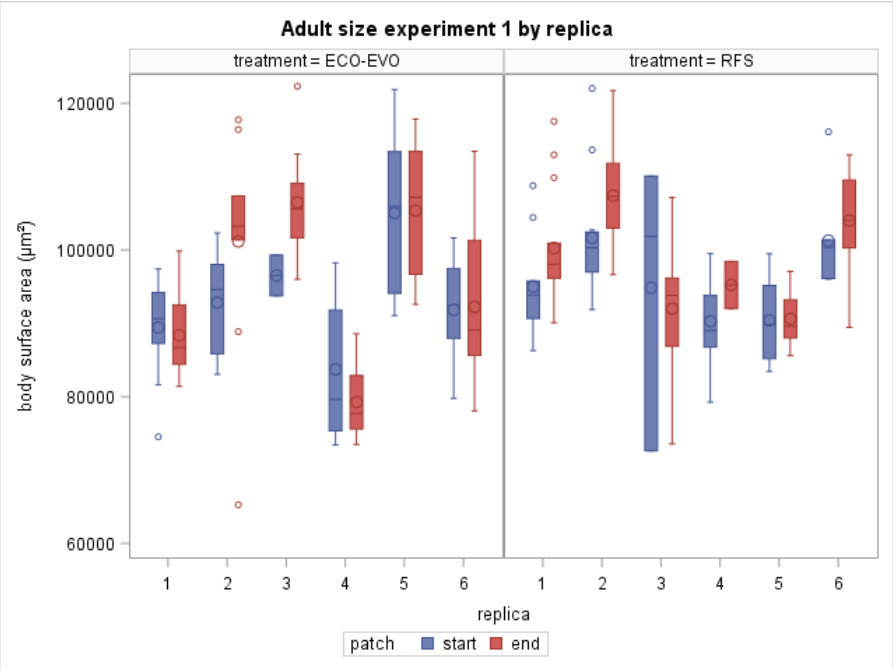

Fig. 15: Boxplots of adult size by replica and patch for experiment 1

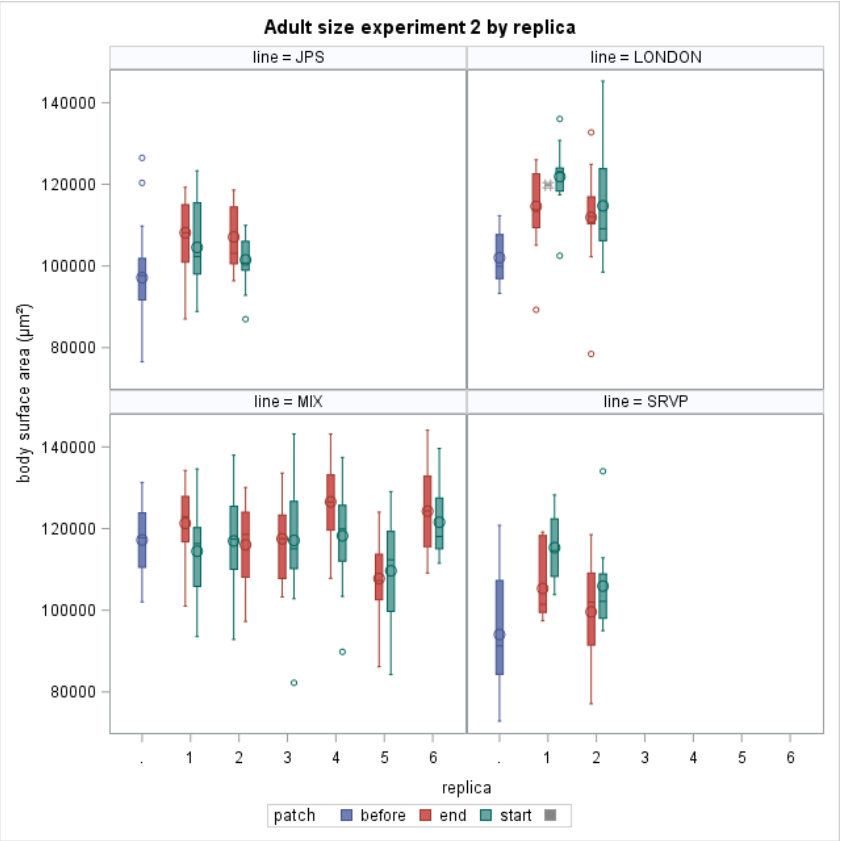

Fig. 16: Boxplots of adult size by replica and patch for experiment 2

Lifetime fecundity

There were no significant differences in lifetime fecundity between the core populations or the populations from the dispersal front in any of the treatments of both experiments.

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,5}$=0.06; p=0.8118	$F_{1,4}$=1.69; p=0.2633
Experiment 2 ECO-EVO2-ECO	$F_{1,3.85}$=0.13; p=0.7398	$F_{1,101}$=0.04; p=0.8456

Table 8: Statistical output for comparison of populations from the core and dispersal front of both experiments, for lifetime fecundity

Mean daily fecundity

Mean daily fecundity did not differ between population from the core or dispersal front in either the evolutionary constrained or unconstrained treatments of both experiments. There did however appear to be again large differences between replicas, as can be seen in the boxplots (Fig. 17-18).

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 **ECO-EVO1 - RFS**	$F_{1,5.21}$=4.42; p=0.0872	$F_{1,5.04}$=1.56; p=0.2666
Experiment 2 **ECO-EVO2-ECO**	$F_{1,5.62}$=0.04; p=0.8458	$F_{1,6.15}$=3.42; p=0.1129

Table 9: Statistical output for comparison of populations from the core and dispersal front of both experiments, for mean daily fecundity

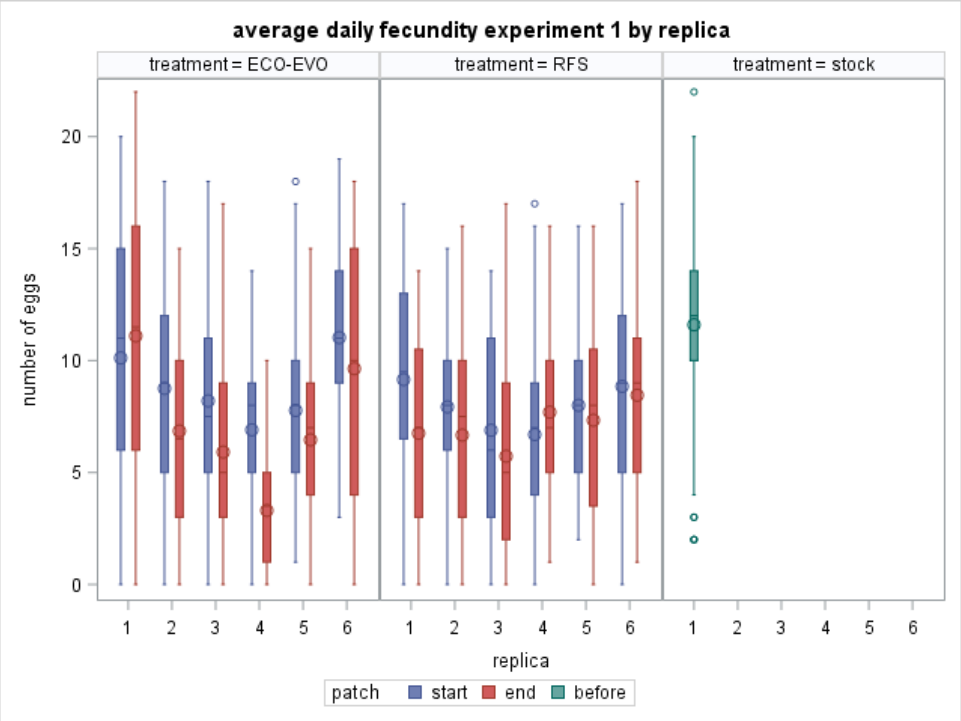

Fig. 17: Boxplots for mean daily fecundity by replica and patch for experiment 1

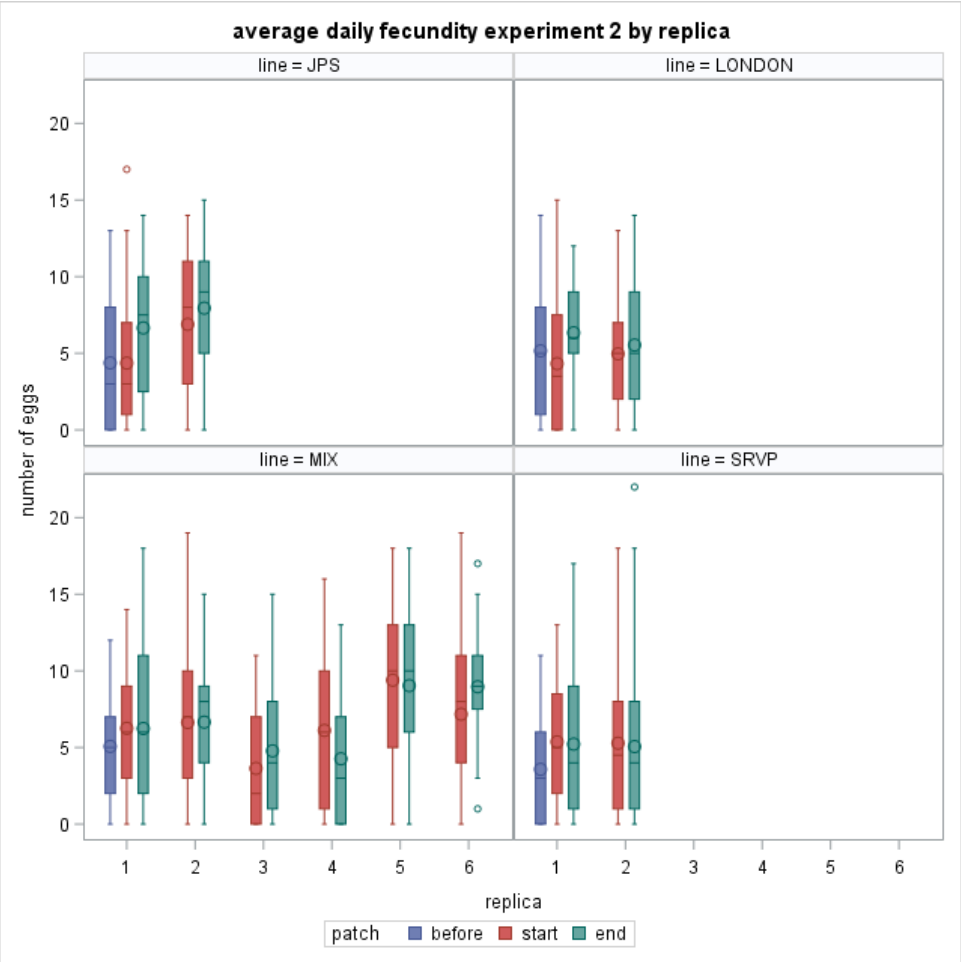

Fig. 18: Boxplots for mean daily fecundity by replica and patch for experiment 2

Cumulative fecundity

No differences could be detected in cumulative fecundity between core and dispersal front populations for the evolutionary constrained and unconstrained treatments in either of both experiments. For the evolutionary constrained treatment of the first experiment (RFS), there was a significant difference in cumulative density increase over time, but this was not consistent over both experiments.

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	Slope: $F_{1,668}$=0.20; p=0.6575 Intercept: $F_{1,9.95}$=0.20; p=0.6624	**Slope: $F_{1,641}$=5.33; p=0.0212** Intercept: $F_{1,11.6}$=1.69; p=0.2192
Experiment 2 ECO-EVO2-ECO	Slope: $F_{1,1131}$=0.13; p=0.7194 Intercept: $F_{1,10.1}$=0.14; p=0.7116	Slope: $F_{1,1121}$=0.02; p=0.9023 Intercept: $F_{1,11.3}$=0.190; p=0.6704

Table 10: Statistical output for comparison of populations from the core and dispersal front of both experiments, for cumulative fecundity

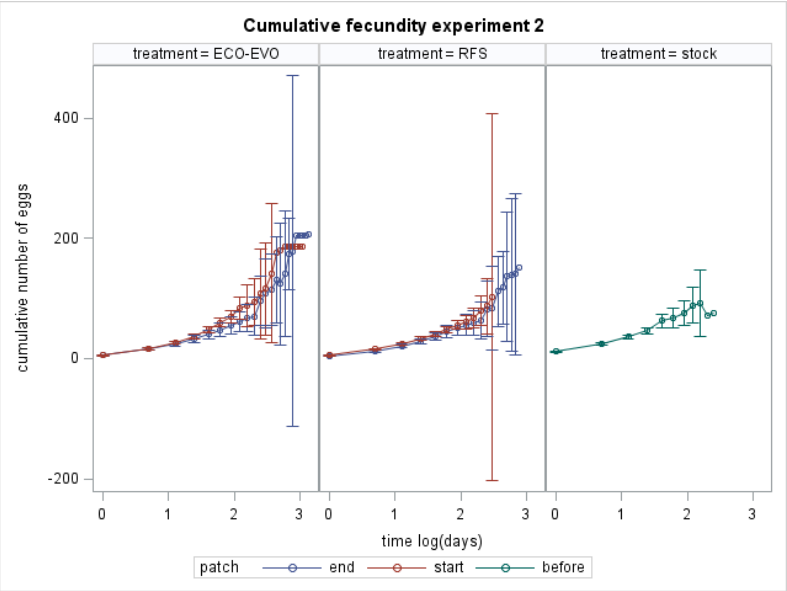

Fig. 19: Cumulative fecundity by treatment and patch for experiment 1. Flags represent confidence limits.

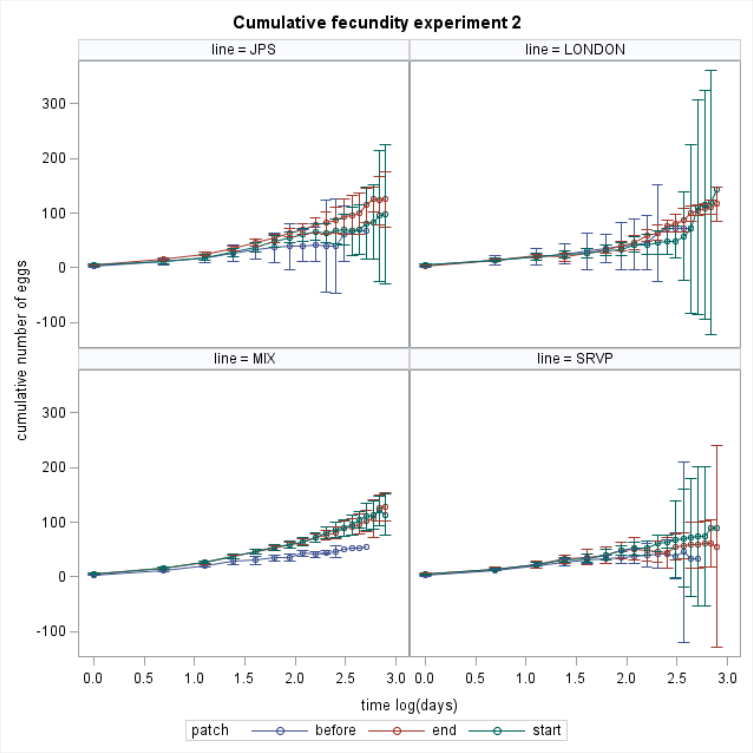

Fig. 20: Cumulative fecundity by treatment and patch for experiment 2. Note that data has been limited up to 18 days, as error bars became too large after that time, due to small number of females remaining, hindering interpretation of the plot. Flags represent confidence limits.

Longevity

Longevity did not differ between populations from the core and the dispersal front in neither the evolutionary unconstrained and constrained treatments of both experiments (see table 11 for statistical output).

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,10}$=1.15; p=0.3091	$F_{1,4}$=0.13; p=0.7382
Experiment 2 ECO-EVO2-ECO	$F_{1,5.33}$=0.16; p=0.7039	$F_{1,103}$=0.54; p=0.4635

Table 11: Statistical output for comparison of populations from the core and dispersal front of both experiments, for longevity

Leaf consumption

No clear trends could be found in differences between populations from the core and the dispersal front, in terms of leaf consumption. Statistical analyses only yielded one significant difference, for the evolutionary unconstrained treatment of experiment 2 (ECO-EVO2) where leaf consumption after 3 days was significantly lower at the dispersal front compared to the core population (**$F_{1,64}$=4.11; p=0.0469**). Note however that the result is only marginally significant, which (in combination with the absence of similar trends after 5 days, or in the first experiment) suggests this is no consistent relation. It should be noted that the protocol to calculate leaf consumption fails to work well on some pictures, resulting in an overestimation of leaf consumption, and therefore the results of leaf consumption should be interpreted with the necessary caution. It may be possible that there do occur adaptive responses in leaf consumption, which we may fail to pick up on due to these limitations in the used method of analysis.

Core vs. dispersal front (3days)	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,3.88}$=0.49; p=0.5244	$F_{1,67.7}$=0.82; p=0.3689
Experiment 2 ECO-EVO2-ECO	**$F_{1,64}$=4.11; p=0.0469**	$F_{1,10.2}$=0.03; p=0.8709

Table 12: Statistical output for comparison of populations from the core and dispersal front of both experiments, for leaf consumption after 3 days

Core vs. dispersal front (5days)	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,49.9}$=1.72; p=0.1960	$F_{1,1.66}$=0.03; p=0.8721
Experiment 2 ECO-EVO2-ECO	$F_{1,3.95}$=1.03; p=0.3685	$F_{1,8.75}$=1.79; p=0.2152

Table 13: Statistical output for comparison of populations from the core and dispersal front of both experiments, for leaf consumption after 5 days

Ambulatory dispersal

Mean distance moved was significantly different between the populations from the core and dispersal front of the evolutionary constrained treatment of the second experiment (ECO), however consistent trends in dispersal behaviour were lacking.

Core vs. dispersal front	Evolutionary unconstrained	Evolutionary constrained
Experiment 1 ECO-EVO1 - RFS	$F_{1,10}$=0.07; p=0.7956	$F_{1,10}$=0.27; p=0.6145
Experiment 2 ECO-EVO2-ECO	$F_{1,17}$=2.58; p=0.1263	**$F_{1,17}$=4.91; p=0.0406**

Table 14: Statistical output for comparison of populations from the core and dispersal front of both experiments, for mean distance moved at day 4

Drift assessments

Development time

For both male and female development time of the evolutionary unconstrained metapopulations (ECO-EVO1 and ECO-EVO 2), there is a consistent trend towards a significant effect of replica. This indicates there is a strong effect of initial population composition, or of serious drift effects early on during the range expansion experiment. In case of the second experiment, there is a strong effect of within replica response (replica*patch interaction) as well, indicating that stochastic effects during the course of the range expansion experiment shape phenotypic variation as well. Note that in the first experiment these within replica responses are only marginally non-significant for male development time as well. In the evolutionary constrained treatments (RFS and ECO), no such consistent patterns emerge, with only replica significantly affecting male development time in the first experiment, and a significant effect of within replica response on female development time for the second experiment. There are however no clear trends in drift effects, as was the case for the evolutionary unconstrained treatments.

Experiment 1 Females	ECO-EVO1	RFS
Replica	**$F_{5,178}$=7.97; p<0.0001**	$F_{5,161}$=0.71; p=0.6169
Replica*patch	$F_{5,178}$=1.28; p=0.2761	$F_{5,161}$=0.31; p=0.9038

Table 15: Statistical output of drift assessments for female development time of experiment 1

Experiment 1 Males	ECO-EVO1	RFS
Replica	**$F_{5,122}$=4.59; p=0.0007**	**$F_{5,135}$=8.52; p<0.0001**
Replica*patch	$F_{5,122}$=2.27; p=0.0511	$F_{5,135}$=1.16; p=0.3340

Table 16: Statistical output of drift assessments for male development time of experiment 1

Experiment 2 Females	ECO-EVO2	ECO
Replica	**$F_{5,245}$=12.04; p<0.0001**	$F_{3,149}$=1.40; p=0.2391
Replica*patch	**$F_{5,245}$=10.45; p<0.0001**	**$F_{5,145}$=2.28; p=0.0496**

Table 17: Statistical output of drift assessments for female development time of experiment 2

Experiment 2 Males	ECO-EVO2	ECO
Replica	**$F_{5,162}$=3.26; p=0.0078**	$F_{3,176}$=1.53; p=0.2085
Replica*patch	**$F_{5,162}$=2.87; p=0.0164**	$F_{5,175}$=2.05; p=0.0739

Table 18: Statistical output of drift assessments for male development time of experiment 2

Sex ratio

Whereas there were some significant drift effects (within replica response in the first experiment, and replica in the ECO-EVO2 treatment of the second experiment), no clear patterns can be observed, as results of both experiments indicate the occurrence of different drift effects.

Experiment 1	ECO-EVO1	RFS
Replica	$F_{5,48.17}$=0.79; p=0.5630	$F_{5,48.19}$=0.49; p=0.7818
Replica*patch	**$F_{5,48.17}$=2.92; p=0.0220**	**$F_{5,48.19}$=2.49; p=0.0439**

Table 19: Statistical output of drift assessments for sex ratio of experiment 1

Experiment 2	ECO-EVO2	ECO
Replica	**$F_{5,154}$=2.87; p=0.0166**	$F_{3,145}$=1.22; p=0.3033
Replica*patch	$F_{5,154}$=0.67; p=0.6498	$F_{5,145}$=0.47; p=0.8564

Table 20: Statistical output of drift assessments for sex ratio of experiment 2

Adult size

Replica had a significant effect on adult size in both treatments of the first experiment (ECO-EVO1 and RFS) and the evolutionary unconstrained treatment of the second experiment (ECO-EVO2). Drift associated with within replica response did not occur in any of the treatments. The occurrence of drift effects associated with replica in the evolutionary unconstrained treatments may indicate initial differences in population composition at the start of the range expansion experiment. However, the strong effect of replica that is present in the RFS treatment cannot be explained by these causes, as the entire metapopulation (during the last replacements approximately 200-300 females) was still replaced two weeks prior to collection.

Experiment 1	ECO-EVO1	RFS
Replica	**$F_{5,122}$=21.17; p<0.0001**	**$F_{5,122}$=12.03; p<0.0001**
Replica*patch	$F_{5,122}$=1.52; p=0.1869	$F_{5,122}$=0.80; p=0.5510

Table 21: Statistical output of drift assessments for adult size of experiment 1

Experiment 2	ECO-EVO2	ECO
Replica	**$F_{5,204}$=10.18; p<0.0001**	$F_{3,123}$=2.12; p=0.1016
Replica*patch	$F_{5,204}$=1.43; p=0.2157	$F_{5,123}$=1.75; p=0.1290

Table 22: Statistical output of drift assessments for adult size of experiment 2

Lifetime fecundity

In the evolutionary unconstrained treatments of both the first range expansion experiment (ECO-EVO1) and the second range expansion experiment (ECO-EVO2), there were significant drift effects associated with replica. There were no differences in within replica response, nor did the evolutionary constrained setups show any indication of drift effects associated with replica. As before, this suggests a strong effect of initial population composition or early drift effects.

Experiment 1	ECO-EVO1	RFS
Replica	**$F_{5,96}$=3.47; p=0.0063**	$F_{5,92}$=0.51; p=0.7717
Replica*patch	$F_{5,96}$=1.17; p=0.3288	$F_{5,92}$=0.27; p=0.9289

Table 23: Statistical output of drift assessments for lifetime fecundity of experiment 1

Experiment 2	ECO-EVO2	ECO
Replica	**$F_{5,82.3}$=8.06; p<0.0001**	$F_{3,93}$=0.04; p=0.9880
Replica*patch	$F_{5,82.3}$=1.74; p=0.1344	$F_{5,93}$=0.41; p=0.8377

Table 24: : Statistical output of drift assessments for lifetime fecundity of experiment 2

Mean daily fecundity

In the evolutionary unconstrained treatments of experiment 1 (ECO-EVO1) and experiment 2 (ECO-EVO2), there was a significant effect of both replica and within replica response on mean daily fecundity. There was however a significant effect of replica as well in the evolutionary constrained treatment of experiment 1 (RFS) and both of replica and within replica response for experiment 2 (ECO). Therefore it is not possible to state that variability between samples has a genetic nature, as there appears to be strong variation in the evolutionary constrained treatments. Likely, variability is shaped by plasticity however the cause of divergence between and within different metapopulations cannot be determined from our study design.

Experiment 1	ECO-EVO1	RFS
Replica	**$F_{5,564}$=20.25; p<0.0001**	**$F_{5,555}$=3.26; p=0.0065**
Replica*patch	**$F_{5,564}$=2.73; p=0.0188**	$F_{5,555}$=1.73; p=0.1250

Table 25: Statistical output of drift assessments for mean daily fecundity of experiment 1

Experiment 2	ECO-EVO2	ECO
Replica	**$F_{5,986}$=23.68; p<0.0001**	**$F_{3,928}$=6.54; p=0.0002**
Replica*patch	**$F_{5,986}$=2.61; p=0.0237**	**$F_{5,923}$=2.44; p=0.0330**

Table 26: Statistical output of drift assessments for mean daily fecundity of experiment 2

Longevity

In the evolutionary constrained treatments of both the first experiment (RFS) and the second experiment (ECO), no drift effects could be detected. For the evolutionary unconstrained treatments, results are not consistent. Whereas neither replica or within replica response has a significant effect on longevity in the first experiment (ECO-EVO1), both are significant in the second experiment (ECO-EVO2).

Experiment 1	ECO-EVO1	RFS
Replica	$F_{5,96}$=0.74; p=0.5959	$F_{5,92}$=0.16; p=0.8882
Replica*patch	$F_{5,96}$=1.49; p=0.2011	$F_{5,92}$=0.34; p=0.9748

Experiment 2	ECO-EVO2	ECO
Replica	**$F_{5,83.3}$=3.21; p=0.0106**	$F_{3,93}$=1.21; p=0.3093
Replica*patch	**$F_{5,83.3}$=2.95; p=0.0169**	$F_{5,93}$=0.47; p=0.8003

Leaf consumption

There appears to be a consistent trend of significant drift effects associated with replica, indicating a strong effect of initial population composition, or early drift effects on leaf consumption. For the evolutionary unconstrained treatment of the first experiment however, there are no significant drift effects (either of replica or within replica response). Note that in the evolutionary constrained treatment of the second experiment ECO), replica is only marginally non-significant on leaf consumption after 5 days. For the second experiment, within replica response had a significant effect on leaf consumption both after 3 days and 5 days in the evolutionary constrained (ECO) and unconstrained (ECO-EVO1) treatments, however this trend did not appear in the first experiment. As stated above, the results of leaf consumption analysis should be interpreted with some caution, due to limitations in the analysis method.

Experiment 1: 3 days	ECO-EVO1	RFS
Replica	$F_{5,53.5}$=1.25; p=0.2977	**$F_{5,60}$=3.59; p=0.0066**
Replica*patch	$F_{5,53.5}$=0.72; p=0.6107	$F_{5,60}$=0.68; p=0.6435

Table 27: Statistical output of drift assessments for leaf consumption after 3 days for experiment 1

Experiment 1: 5 days	ECO-EVO1	RFS
Replica	$F_{5,38.6}$=1.24; p=0.3097	**$F_{5,30.7}$=2.66; p=0.0413**
Replica*patch	$F_{5,38.6}$=0.46; p=0.8056	$F_{4,30.4}$=1.13; p=0.3610

Table 28: Statistical output of drift assessments for leaf consumption after 5 days for experiment 1

Experiment 2: 3 days	ECO-EVO2	ECO
Replica	**$F_{5,95.5}$=5.63; p=0.0001**	**$F_{3,96}$=2.72; p=0.0487**
Replica*patch	**$F_{5,95.5}$=2.59; p=0.0305**	**$F_{5,96}$=2.96; p=0.0158**

Table 29: Statistical output of drift assessments for leaf consumption after 3 days for experiment 2

Experiment 2: 5 days	ECO-EVO2	ECO
Replica	**$F_{5,99}$=5.20; p=0.0003**	$F_{3,92}$=2.68; p=0.0517
Replica*patch	**$F_{5,99}$=2.96; p=0.0157**	**$F_{5,92}$=5.10; p=0.0004**

Table 30: Statistical output of drift assessments for leaf consumption after 5 days for experiment 2

Ambulatory dispersal

Drift effects could not be tested for the first experiment, however visual interpretation of the plots indicates drift in response between replicas may be of importance for the ECO-EVO1 treatment (Fig. 21), but not in the RFS treatment (Fig. 22). In half of the replicas (replica 1-3) for the ECO-EVO1 treatment, mean distance moved is higher in the end patches compared to the start patches, whereas for the other half (replica 4-6), the opposite is the case. Relative distances moved for start and end patches within each replica are strikingly consistent during the entire week. For the RFS treatment however, no such patterns can be seen in the week, and relative distance for start and end patches within each replica are not consistent, but change regularly. Drift assessment for the second experiment did not yield any significant results, nor did the graphs show clear trends (see appendix), however this may again be due to limited sample size per replica (two transects for every start and end patch of each replica).

Experiment 2	ECO-EVO2	ECO
Replica	$F_{5,12}$=2.24; p=0.1167	$F_{3,12}$=0.18; p=0.9064
Replica*patch	$F_{5,12}$=0.89; p=0.5167	$F_{5,12}$=0.38; p=0.8479

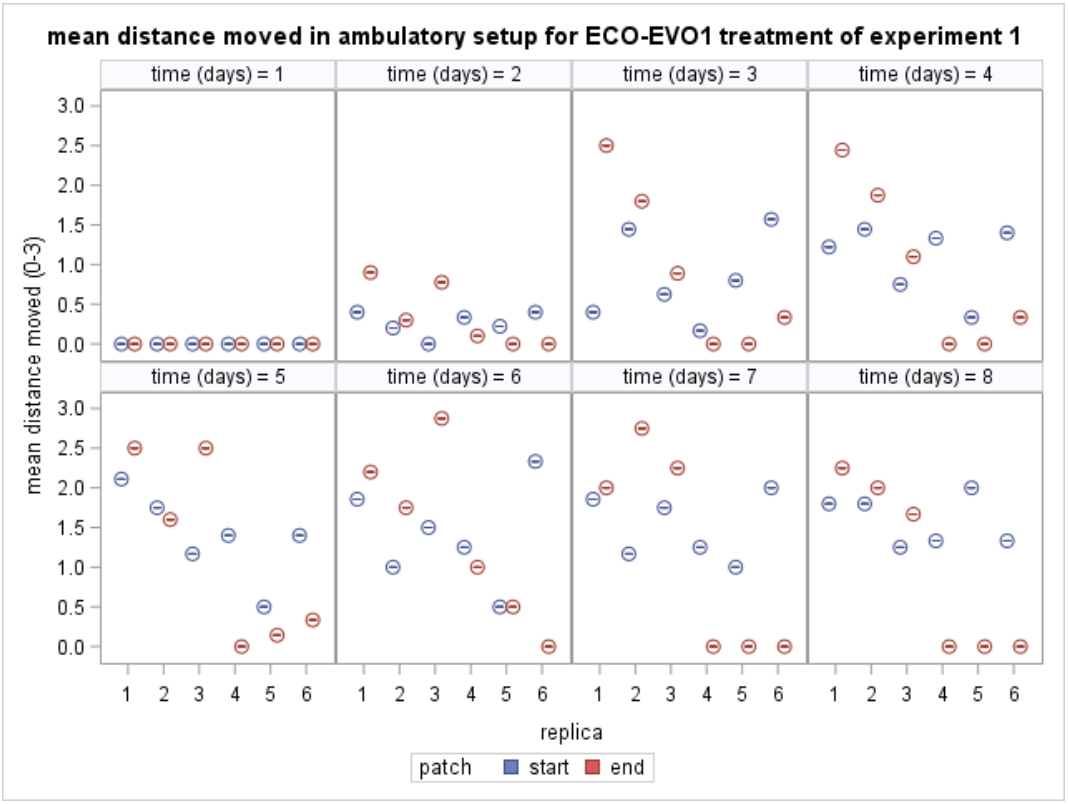

Fig. 21: Mean distance moved on ambulatory dispersal setups for start and end patches of all replicas for the ECO-EVO1 treatment of experiment 1

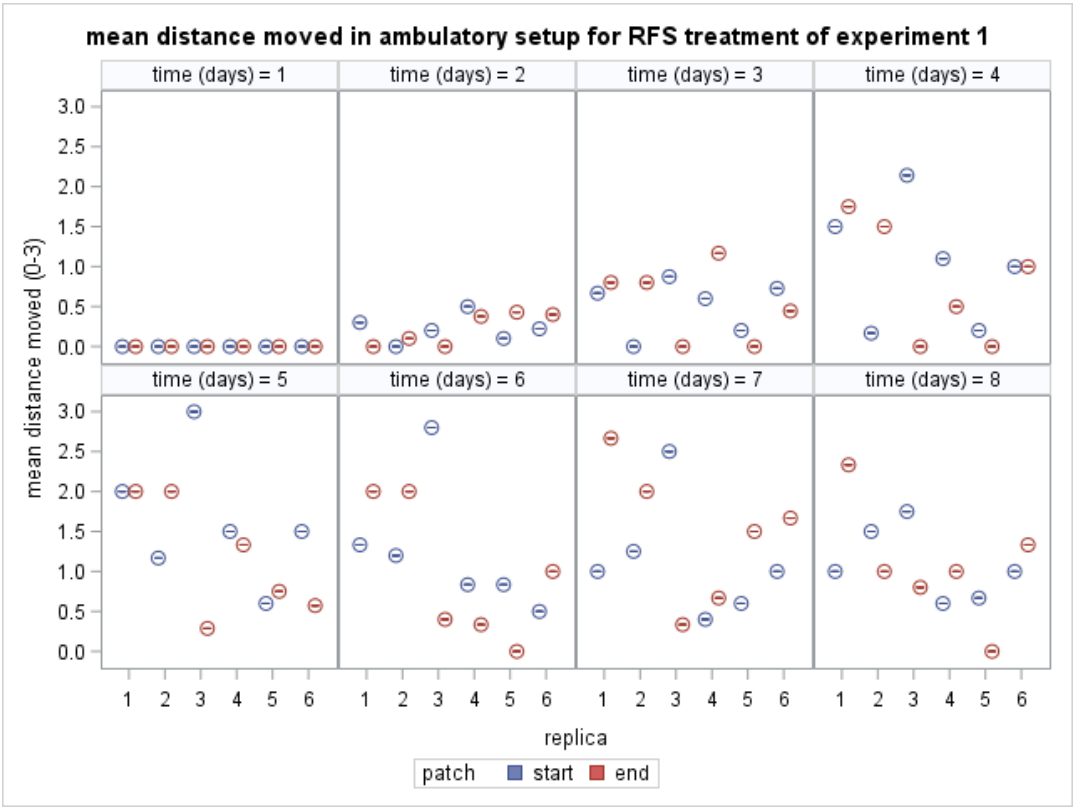

Fig. 22: Mean distance moved on ambulatory dispersal setups for start and end patches of all replicas for the RFS treatment of experiment 1

	ECO-EVO1/2: replica	ECO-EVO1/2: within replica response	RFS/ECO: replica	RFS/ECO: within replica response
Development time	Consistent across experiments	Inconsistent across experiments	Absent	Absent
Sex ratio	Absent	Absent	Absent	Absent
Adult size	Consistent across experiments	Absent	Inconsistent across experiments	Absent
Lifetime fecundity	Consistent across experiments	Absent	Absent	Absent
Mean daily fecundity	Consistent across experiments	Consistent across experiments	Consistent across experiments	Inconsistent across experiments
Longevity	Inconsistent across experiments	Inconsistent across experiments	Absent	Absent
Leaf consumption	Inconsistent across experiments	Inconsistent across experiments	Inconsistent across experiments	Inconsistent across experiments
Ambulatory dispersal	Absent	Absent	Absent	Absent

Table 31: Overview of drift effects, with indication if drift effects related to either replica or within replica response occurred consistently across the two range expansion experiments (green), inconsistently (only in one experiment, orange) or were lacking completely (red) in the evolutionary unconstrained (ECO-EVO1 and ECO-EVO2) or constrained (RFS and ECO) treatments.

Multivariate analysis

No clear clustering could be observed of start and end patches of replicas could be observed for the first experiment (Fig. 24). For the second experiment (Fig. 25) however, variability appears to be lower for end patches of the ECO-EVO2 treatment (MIX line), with all samples (with the exception of MIX4 end) clustering in the lower left quadrant of the ordination plot. In order to determine the main traits driving divergence between sample means, the five most influential traits were depicted on the ordination plots. Variability between samples was mainly driven by differences in fecundity (mean daily fecundity, lifetime fecundity and cumulative fecundity) and leaf consumption (both after three and five days), for both experiments. As for none of these traits, there were clear signs of adaptive responses acting according to start or end patch, these results may again be indicative of the importance of drift (both between and within replicas) driving variability. For both experiments, samples from the core have been connected with their counterpart from the dispersal front by means of an arrow (green for the evolutionary constrained treatments, orange for the unconstrained treatments). As such, we can try and detect whether shift occur along a specific direction or not. As can be seen in both

graphs, shifts occur for the largest part in a non-directional way, in the sense that shifts are not in one particular direction according to treatment. In fact, several shifts occur in opposite directions. In case of the evolutionary constrained treatments, shift occur however mostly along the axes of leaf consumption, whereas for the unconstrained treatments, shifts occur both along the axes of leaf consumption, as well as the axes of fecundity. Thus there appears to be a lot of variation in leaf consumption, through plasticity, and some shifts in fecundity based on evolutionary processes. The lack of one direction in which these evolutionary process occur again suggests drift plays a large role in shaping the phenotypic variation at the dispersal front.

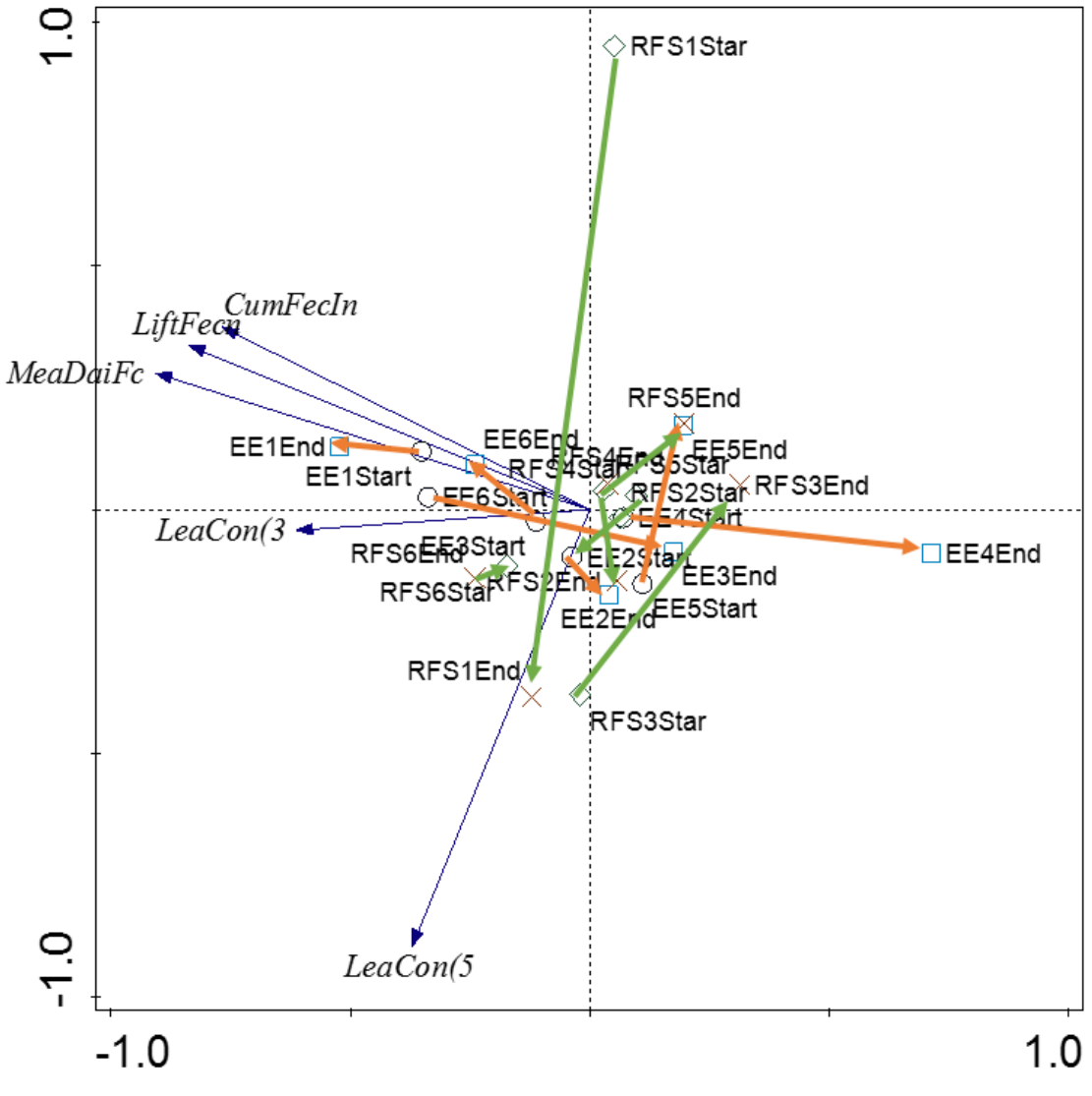

Fig. 23: Ordination plot of experiment 1 with samples and five most influential traits. LeaCon(5=leaf consumption after five days, LeaCon3(=leaf consumption after 3 days, MeaDaiFc=mean daily fecundity, LiftFecn=Lifetime fecundity and CumFecIn=cumulative fecundity. Orange arrows connect core populations of the ECO-EVO1 treatment with their dispersal front counterparts and green arrows do the same for the RFS treatment.

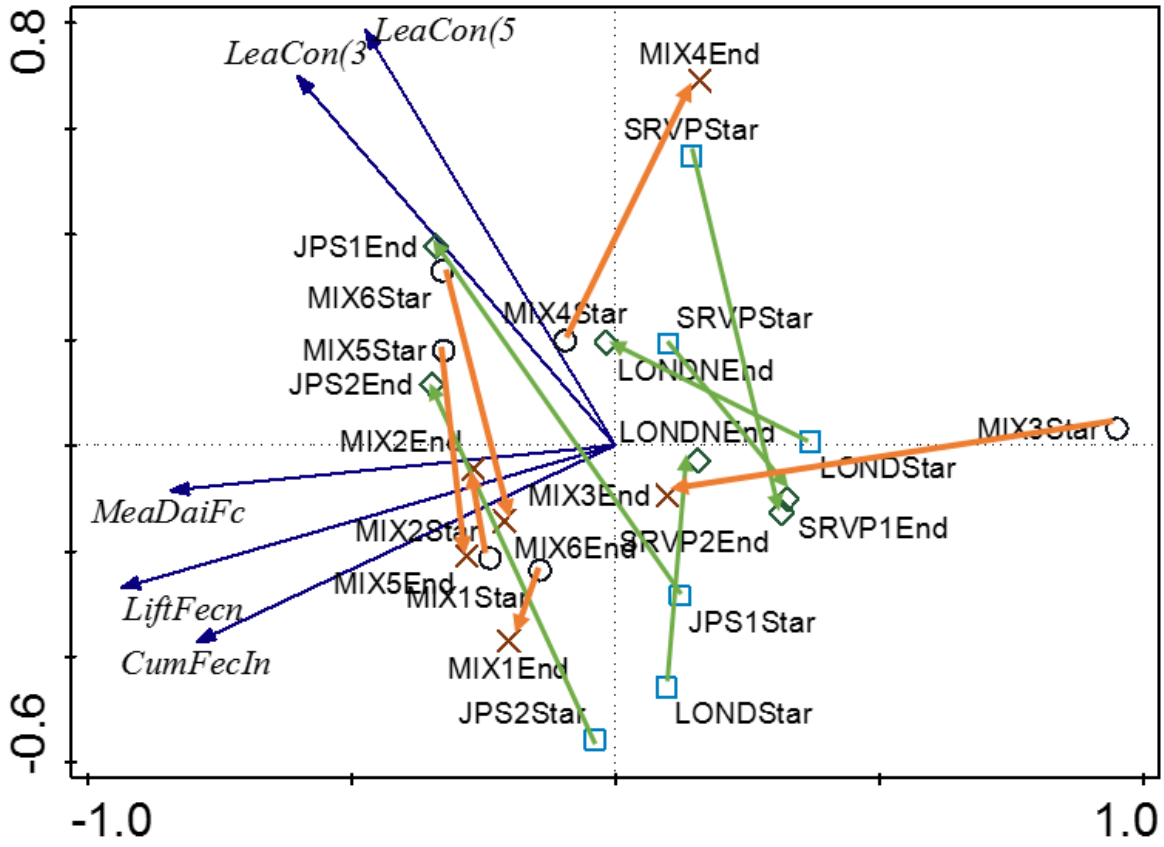

Fig. 24: Ordination plot of experiment 2 with samples and five most influential traits. LeaCon(5=leaf consumption after five days, LeaCon3(=leaf consumption after 3 days, MeaDaiFc=mean daily fecundity, LiftFecn=Lifetime fecundity and CumFecIn=cumulative fecundity. Orange arrows connect core populations of the ECO-EVO2 treatment with their dispersal front counterparts and green arrows do the same for the ECO treatment.

Discussion

In both of our experiments, range expansion occurred faster in the evolutionary unconstrained treatments, compared to the constrained treatments, indicating that spatial selection leads to an increase of range expansion rate. This appears to be in accordance with previous findings, that evolution accelerates range expansion (Phillips et al., 2010a). There is however a strong difference between the two experiments, both in experimental design and in range expansion results. In the first experiment, individuals from the evolutionary constrained setup (RFS) were replaced starting from a week after initiation of range expansion, resulting in overall low relatedness at the dispersal front, whereas in the evolutionary unconstrained setup (ECO-EVO1), relatedness is expected to be high, due to small numbers of individuals colonising the patches, and subsequently procreating until density rises again. During the second range

expansion however, both evolutionary unconstrained (ECO-EVO2) and constrained (ECO) were not manipulated. Relatedness was thus not altered weekly as was the case in the first experiment, and no relatedness induced differences in dispersal rate could occur between the evolutionary constrained and unconstrained treatments in the second experiment, and thus drive differences in range expansion. In the first experiment, where both spatial selection was inhibited, and kin structure was destroyed in the evolutionary constrained treatment, the difference in length of the metapopulation was approximately six patches at the end of the range expansion experiment. In the second experiment, where kin structure was maintained, range expansion was still faster in the unconstrained setup, but the difference was only approximately one patch. This suggests that whereas range expansion does lead to accelerating range expansion through spatial selection, transgenerational plasticity associated with increased relatedness at the dispersal front has a far stronger effect than spatial selection. Consequently, our results fall in line with predictions from theoretical modelling, which indicated that repeated founder effects at the dispersal front can indeed lead to high relatedness, and consequently rapid acceleration in range expansion (Kubisch, Fronhofer, Poethke *et al.*, 2013). This acceleration in range expansion could come to rise in several ways, including evolution of dispersal behaviour, and population dynamics. In order to try and determine the underlying mechanism, the trends in traits related to dispersal and life history are discussed in the next sections.

Low density conditions at the dispersal front are expected to lead to selection to a more r-selected life history strategy (Chuang & Peterson, 2016; Phillips, Brown & Shine, 2010) as has been demonstrated both by theoretical modelling approaches of range expansion (Alex Perkins, Phillips, Baskett et al., 2013; Burton, Phillips & Travis, 2010b) and field studies performed on species which have expanded their range (Amundsen et al., 2012; Therry et al., 2014b). In accordance with these expectations, the results of our experiment indicate that rapid adaptations in life history traits can indeed occur during periods of range expansion. After ten generations, population growth rates were significantly higher at the dispersal front, compared to the core in the evolutionary unconstrained treatments of both range expansion experiments. Seen as there were no significant differences in the evolutionary constrained setups, divergence between the dispersal front and core populations in terms of population growth rate is likely the result of adaptive evolution caused by the continuous colonisation of empty habitat and the consequential fitness advantage of fast reproducers and not caused by transgenerational plasticity induced by the lower density conditions experienced at the dispersal front. The increase in population growth at the dispersal front could arise through various adaptations. Either an increase in reproductive output (Amundsen, Salonen, Niva et al., 2012; Therry, Bonte & Stoks, 2015), earlier sexual maturation (Sanford, Holzman, Haney

et al., 2006; Therry, Lefevre, Bonte et al., 2014; Therry, Nilsson-Oertman, Bonte et al., 2014) (i.e. development time in our experiments) or an increased proportion of offspring reaching the adult stage (egg and juvenile survival for our experiment) could possibly explain the observed increase in population growth rate. As we did not find any clear differences between populations from the core and dispersal front in the evolutionary unconstrained treatments, it is not possible to designate one of the traits as the one altered by selection at the dispersal front, resulting in increased population growth. Whereas in the field study of the two-spotted spider mite by Van Petegem et al. (Van Petegem, Boeye, Stoks et al., 2015) a significant decrease in development time was discovered, such a response is completely lacking in our results, which is in accordance with their conclusion that local adaptation associated with changes in length of growing season (and linked changes in voltinism) rather than due to spatial selection during range expansion. One possible explanation for the lack of clear responses in the individual life history traits is that the nature of adaptation towards increased population growth rates is more complex, and governed by multiple traits rather than a single one, resulting in the failure to detect clear differences. A second possibility, is that selection to faster population growth rates arose through different ways in different metapopulations (for instance reduced development time at the dispersal front of some metapopulations, increased fecundity in other metapopulations, etc.). As such, there could be multiple responses leading to the same outcome (‘many paths lead to Rome”). In order to test whether this is the case, the data of the individuals life history traits (fecundity, survival and development time) of the different metapopulations will in the near future be resampled, to calculate the theoretical, density independent population growth rate for every metapopulation. These theoretical population growth rates can then be compared between populations of the core and dispersal front, to assess whether the combined effect of the individual life history traits does indeed lead to increased population growth rates.

Apart from adaptation towards a more r-selected life history, evolution towards increased dispersal ability at the dispersal front was expected as well due to spatial selection according to dispersal ability (Kubisch, Hovestadt & Poethke, 2010; Phillips, Brown, Travis *et al.*, 2008; Shine, Brown & Phillips, 2011), which could explain the acceleration in range expansion observed in both experiments. However, whereas we did find a clear adaptation towards an increased population growth rate at the dispersal front, such clear patterns in mean dispersal distance for either of both experiments, could not be discerned. The lack of such trends might suggest a lack of selection on dispersal ability, thus indicating that dispersal behaviour is mainly shaped by phenotypic plasticity associated with experienced conditions rather than directly through genetic differences between individuals. Experienced density has been identified as a major determining factor for dispersal of the two-spotted spider mite (Bitume,

Bonte, Magalhaes et al., 2011; Bitume, Bonte, Ronce et al., 2013; Bitume, Bonte, Ronce et al., 2014). This would however imply that only direct density conditions affect dispersal, as a single generation under common garden conditions was sufficient to negate all possible effects of density conditions. This assumption is rather unlikely, as past experiments have already indicated maternal effects (Bitume, Bonte, Magalhaes et al., 2011) and more specifically the experienced density conditions by both the parent and grandparent populations (Bitume, Bonte, Magalhaes et al., 2011; Bitume, Bonte, Ronce et al., 2014) can strongly affect dispersal behaviour.

For both range experiments, populations from the evolutionary unconstrained setups did however expand their range significantly faster compared to the constrained setups. As density conditions were not influenced (by means of intervention) in the evolutionary constrained treatments, this seems to contradict the lack of differences found for mean dispersal distance, and to suggest that dispersal behaviour is affected by other factors than experienced density conditions itself. Several possible mechanisms could be shaping dispersal behaviour itself. A possible explanation would be there is indeed no selection on dispersal behaviour itself, and the faster range expansion in the evolutionary constrained treatments is caused by the combination of selection for faster population growth at the front, and dispersal behaviour being dependent on density conditions. As demonstrated above, spatial selection led to increased population growth rates at the dispersal front of evolutionary unconstrained treatments. As population sizes at the dispersal front of the evolutionary unconstrained setups would over time have increased faster compared to the evolutionary unconstrained setups, theoretically this should lead to faster range expansion in the unconstrained setups. This mechanism can, in combination with the strongly contributing effect of relatedness (and thus kin competition) in case of the first experiment, explain the acceleration in range expansion of evolutionary unconstrained treatments of the experiments. A second possibility would be the evolution of density dependence of dispersal behaviour along the dispersal behaviour. If selection leads to expression of density dispersal at lower densities compared to the core, this could again lead to the pattern of faster range expansion in the evolutionary unconstrained treatments, compared to the constrained treatments. Modelling approaches have already indicated evolution of density dependence may be important during range expansion (Travis, Mustin, Benton *et al.*, 2009). We can however not make definitive statements yet concerning the evolution of density dependence of dispersal for this lab experiment, as only one density treatment has been included in the experimental design for the assessment of ambulatory dispersal. In order to test the likelihood of evolution of density dependence, I will in the coming months develop an individual based model based on the results of our experiments, in which the evolution of transgenerational plasticity associated with density conditions will be included.

This may help to shed light on the underlying mechanism of range expansion acceleration. A last possibility is that while there may not be a change in mean dispersal distance, there may be changes in the dispersal kernel at the dispersal front, which remain undetected by the used metric. Especially for the first experiment, there appears to be a tendency for larger variability in mean distance moved at the dispersal front of the evolutionary unconstrained treatments compared to the core populations, whereas no such trends can be found for the evolutionary constrained treatment. Past experiments testing the effects of relatedness (Bitume, Bonte, Ronce et al., 2013) used a different experimental setup to assess ambulatory dispersal, in which dispersal was possible over larger differences compared to our setup. It is noted in this article that whereas mean distance moved was only limitedly affected by relatedness, long distance dispersal was strongly dependent on relatedness. Hence our experimental setup for the ambulatory dispersal may have been insufficient to detect effects of long distance dispersal, which, in combination with the relatively small sample sizes, may explain the failure to detect significant differences in dispersal behaviour.

For several of the assessed traits, a distinct pattern can be discerned in terms of drift effects. Overall, strong drift effects associated with replica of the range expansion experiment could be detected, for adult size, development time, lifetime fecundity and mean daily fecundity. For both ambulatory dispersal of the first experiment and lifetime fecundity, there appeared to be strong drift effects for within replica response as well. During periods of range expansion, populations undergo a sequence of colonisation events, which can strongly affect allelic frequencies (Slatkin & Excoffier, 2012). The colonisation events represent in essence a series of bottlenecks (Edmonds, Lillie & Cavalli-Sforza, 2004), resulting in the stochastic loss of alleles, and a typical decrease in heterozygosity (Austerlitz, JungMuller, Godelle et al., 1997; Hallatschek & Nelson, 2008; Slatkin et al., 2012). Furthermore, these stochastic effects in allele frequencies during range expansion can, in case new neutral mutations arise, lead to surfing of these new alleles with the dispersal front during further range expansion, as the low density conditions and subsequent bottlenecks allow for relatively fast spread of these mutations (Hallatschek & Nelson, 2008; Edmonds, Lillie & Cavalli-Sforza, 2004; Klopfstein, Currat & Excoffier, 2006). Due to these very swift and strong drift effects, even deleterious alleles can become dominant at the dispersal front through mutation surfing. Our experimental setup, consisting of several distinct patches rather than a continuous habitat, can perhaps best be compared with serial founder effect models, in which multiple subsequent colonisations occur, each representing a separate founder event as described in the model by Peter et Slatkin (Peter & Slatkin, 2015). Considering the theory surrounding loss of genetic variation during range expansion, one can mainly expect drift to cause divergence between the dispersal fronts of different replicas (i.e. within replica response), however, we see mostly consistent trends

towards differentiation between the replicas. This may be a consequence of the experimental setup, in which ten inseminated females are collected from the initial stock population, and placed on the first patch of the metapopulation used for the range expansion experiment. In such a setup, there is no longer the possibility of gene flow with the stock population after initiation of the range expansion experiment. Seen as this is also the situation in invasion cases, our results show again the importance of founder effects for invasions. Such stochasticity through initial drift can indeed majorly influence evolution of life history traits at the dispersal front (Williams et al, 2016), and genetic drift due to range expansion has been shown to lead to genetic divergence on a short time scale for a microbial system experiencing range expansion (Hallatschek, Hersen, Ramanathan et al., 2007). It does however not explain why the observed trend occurs as well in the evolutionary constrained treatment of the first experiment, in which a divergence between replicas is observed as well for adult size, leaf consumption and mean daily fecundity. During the RFS treatment, all female adults were replaced on a weekly basis, which should prevent such drift effects from occurring. Nonetheless, even though at the last replacement, total population sizes for each metapopulation were between 200 and 300 individuals, still drift effects associated with replica, rather than within replica response could be observed. Based on the metapopulation structure, one could expect strong drift effects of within replica response, as subpopulations were relatively small, and low densities at the dispersal front could again lead to strong drift effects on a limited time scale. Even though drift effects associated within replica response are rarely consistent across both experiments (which was only the case for mean daily fecundity), there is however a non-consistent trend of drift associated with within replica response for three traits (development time, longevity and leaf consumption). This seems to suggest that range expansion can lead to stochastic loss of genetic variation, seen as responses at the dispersal front can differ between metapopulations. However, as traits subjected to drift effects associated with within replica response differ between experiments, it may be difficult to predict how these drift effects will affect genetic and phenotypic variation at the dispersal front, which may complicate future attempts to try and predict the effects of (climate or otherwise induced) range expansions. The lack of clear drift effects in the evolutionary constrained setup of the second experiment is to be expected, as the lack of standing genetic variation prevented drift to occur altogether. The strong influence of drift effects indicates that evolutionary responses along dispersal fronts may be mainly governed by stochastic evolutionary processes, on traits not affected by spatial selection. The PCA analysis of our data suggests the same, as variability between samples is mainly driven by traits which did not show any indication of being under selection due to the process of range expansion. Both signs of adaptive and neutral evolution have already been demonstrated to occur along dispersal fronts (Swaegers, Mergeay, Van Geystelen et al., 2015) and have been shown to be useful to identify range expansion (Peter

& Slatkin, 2013). Further genetic analyses applied to our experimental approach may prove to be informative in order to complement the phenotypical analysis we have performed with information on loss of genetic variation and heterozygosity at the dispersal front.

	Experiment 1	Experiment 2
Experimental setup	– Evolution constrained by **weekly replacement** of individuals – **Inhibition of spatial sorting** – **Destruction of kin structure**	– Evolution constrained by **use of inbred lines** lacking standing genetic variation – **Inhibition of spatial sorting** – **Kin structure maintained**
Range expansion	– **ECO-EVO1 >>> RFS** – Length of ± 23 patches for ECO-EVO1 – Length of ± 17 patches for RFS – **Spatial sorting and relatedness** (kin competition) drive range expansion differences between treatments – Strong effect of relatedness on range expansion (difference between treatments ≈ 6 patches)	– **ECO-EVO2 > ECO** – Length of ± 22 patches for ECO-EVO2 – Length of ± 21 patches for ECO – **Spatial sorting** drives range expansion difference between treatments – Effect of spatial sorting smaller than effect of relatedness (difference between treatments ≈ 1 patch)
Population growth rate	– Consistent faster increase in population growth at the dispersal front for ECO-EVO1 – No difference for RFS treatment – Spatial selection leads to **increased population growth at dispersal front**	– Consistent faster increase in population growth at the dispersal front for ECO-EVO2 – No difference for ECO treatment – Spatial selection leads to **increased population growth at dispersal front**
Ambulatory dispersal	– Lack of differences in mean distance moved found – Likely regulated through **density and relatedness dependent plasticity** – Experimental setup may not be suited to detect effects of relatedness (Bitume, Bonte, Ronce et al., 2013)	– Lack of differences in mean distance moved found – Likely regulated through **density and relatedness dependent plasticity**
Life history traits (survival, fecundity and development time)	– Lack of clear trends – Possibly **different strategies** in different replicas of range expansion experiment (“Many paths lead to Rome”)	– Lack of clear trends – Possibly **different strategies** in different replicas of range expansion experiment (“Many paths lead to Rome”)

Table 32: Overview of experimental setups, most important responses in both experiments, and possible explanations

Conclusion

Our results clearly show that range expansion can lead to a rapid evolution of life-history strategy. As predicted by theory, spatial selection leads to increased population growth rates at the edge of the dispersal front, due to the advantage of such a life history strategy under the low density conditions experienced at the dispersal front.

Contrary to theory, we did not find any indications for increased ambulatory dispersal propensity or distance, this in contrast with field observations for aerial dispersal of the two-spotted spider mite. Range expansion rate did however did increase in case evolution could occur, and is likely the consequence of increased relatedness at the dispersal front, inducing dispersal through plasticity. Furthermore, selection to increased population growth is likely to contribute to differences in range expansion behaviour, through density induced plasticity.

Evolution of phenotypical traits was strongly driven by drift effects, indicating that stochastic effects majorly influence evolutionary processes during the process of range expansion. In almost all cases, strong founder effects shape or early drift shapes variability between metapopulations. There are however some cases of differences in response within replica, indicating that during range shifts, repeated founder effects can indeed drive stochastic loss of variability. Consequentially, one can expect strong range expansion to lead to changes in species characteristics, possibly affecting ecological and evolutionary processes in the future, and even contributing to speciation.

As both invasions and climate induced range shifts are currently major issues, it will be paramount to better understand both adaptive responses due to spatial sorting, and to anticipate possible consequences of drift and associated loss of genetic diversity, in order to fully understand potential consequences of range shifts on species dynamics and biodiversity.

Summary

The increasing influence of human induced climate change is expected to increasingly threaten the survival of many species. One way species can cope with this increasing pressure is by shifting their range, a phenomenon which has already been found to be occurring for many species.

During range expansion, species continuously expand their range to previously non-colonised habitat through dispersal. As a consequence, the most dispersive individuals are expected to accumulate at the dispersal front through spatial sorting according to dispersal capability. Subsequent assortative mating between highly dispersive individuals is expected to lead to further selection for high dispersal capabilities at the dispersal front. Furthermore, the

individuals undertaking dispersal are not expected to be a random subset of the population, but rather those individuals for whom the costs of dispersal are outweighed by the advantages of moving. In case of range expansion, individuals with a more r-selected life history strategy are expected to experience high fitness gains under the low density conditions experience at the dispersal front, whereas for individuals with a more K-selected life history strategy, fitness gains for moving to non-colonised areas are expected to be far lower. Consequently, spatial selection is expected not only to lead to increased dispersal ability at the dispersal front, but also to selection for a more r-selected life history. Furthermore, populations undergoing range expansion are characterized by a density gradient, with high density at the core, and low density at the dispersal front. Such a density gradient can induce phenotypic plasticity, driving phenotypic divergence between core and dispersal front populations.

During range expansion, the dispersal front advances through a sequence of founder events, in which only a few individuals colonise the new habitat, which has distinct consequences for population structure at the dispersal front. Firstly, due to the repeated founder effects, individuals at the dispersal front tend to be more related to each other, compared to individuals from the population from the core. Secondly, due to the low density at the dispersal front, drift is expected to lead to stochastic loss of genetic variation (and consequently in phenotypic variation) at the dispersal front. As a result, variation at the dispersal front is expected to be shaped jointly by deterministic processes (spatial selection) and stochastic processes (drift due to low population size).

Range expansion has traditionally often been studied in the context of invasion cases, in which a species has rapidly spread in a new region. This kind of field studies often suffers from the drawbacks that environmental conditions may not be constant along the path of range expansion of the focal species. This can complicate interpretation of the results, as distinction between adaptation to local conditions and the effects of spatial selection may not always be apparent. We therefore designed two experiments using the two-spotted spider mite (*Tetranychus urticae*) as a model species, in which we worked under controlled environmental conditions, in order to avoid confounding factors.

The experimental setup consisted of artificially created metapopulations. Our two experiments were designed as to allow evolutionary responses through spatial selection in half of each experiment, and to prevent spatial selection while maintaining density conditions in the other half of both experiments. In the first experiment, evolutionary potential was constrained through the weekly replacement of all female adults with individuals of a stock population. By doing so, effects of spatial selection were inhibited and kin structure of the metapopulation was destroyed on a weekly basis, preventing increased relatedness at the dispersal front. At the

same time, density conditions were not altered. In the second experiment, evolutionary potential was inhibited by the use of inbred lines, lacking standing genetic variation. In this experiment, both kin structure and density conditions were not altered. The combination of both experiments allowed for a relatively complete assessment of all factors (spatial selection, density gradient and increased relatedness) driving phenotypic divergence between populations from the core and from the dispersal front.

In both experiments, we found an acceleration of range expansion in the experimental setups in which evolutionary potential was not constrained, compared to those were evolutionary potential was constrained, indicating that spatial selection results in an increased rate of range expansion over time. The difference in range expansion was however far greater in the first experiments, indicating that while spatial selection does lead to increased range expansion, the effect of high relatedness at the dispersal front is far more important in driving range expansion compared to the process of spatial selection. Furthermore, in both experiments spatial selection did indeed lead to an increased population growth rate at the dispersal front, in accordance with the theory predicting a more r-selected life history strategy at the dispersal front. When assessing the individual life history traits (associated with fecundity, development time and survival) separately, none of the differed showed consistent trends in differences between populations from the core or dispersal front. Possibly, adaptation to faster population growth happened through different mechanisms in different metapopulation, but further analysis is needed to test this theory. We did not find any differences in dispersal behaviour between populations from the core or dispersal front either. Several possibilities, including the evolution of density dependence of dispersal and a non-appropriate metric or method used in the assessment could explain the lack of findings concerning dispersal behaviour.

Analysis of drift effects indicated there is a strong and very consistent initial founder effect, driving variability between different metapopulations. Although less consistent than the initial founder effects, we still found regular drift associated with the low density conditions, resulting in stochastic loss of phenotypic variation at the dispersal front.

Samenvatting

De steeds toenemende invloed van de door mensen veroorzaakt klimaatsverandering vormt in toenemende mate een bedreiging voor het voortbestaan van vele soorten. Eén manier waarop soorten om kunnen gaan met deze toenemende stressor is door hun areaal te verschuiven. Dit soort areaalverbreidingen werd reeds weergenomen bij verscheidene soorten.

Gedurende areaalverbreidingen verbreiden soorten zich continu naar nieuw, nog niet gekoloniseerd habitat. Bijgevolg vindt er een accumulatie plaats van de meest dispersieve individuen aan het dispersie front ten gevolge van ruimtelijke selectie naar dispersie capaciteit. Dit leidt vervolgens tot paring van individuen met individuen die gelijkaardig zijn in dispersiegedrag, wat de selectie voor sterke dispersie capaciteiten aan het dispersie front versterkt. Voorts is het zo dat de individuen die disperseren typisch geen willekeurige groep zijn van de populatie, maar specifiek deze individuen voor wie de kosten van dispersie overtroffen worden door de baten van zich te verplaatsen naar een nieuw habitat. In het geval van areaalverbreiding wordt verwacht dat individuen met een meer r-geselecteerde life history strategie grote fitness voordelen zullen ondervinden onder de lage densiteitscondities aan het dispersie front. Voor individuen met een meer K-geselecteerde life history strategie zijn de verwachtte voordelen van zich te verplaatsen naar een nog niet gekoloniseerd habitat echter veel kleiner. Bijgevolg wordt verwacht dat ruimtelijke selectie leidt tot zowel hogere dispersie capaciteit als een meer r-geselecteerde life history strategie aan het dispersiefront. Tevens is het zo dat het populaties die areaalverbreiding ondergaan, gekarakteriseerd worden door een densiteitsgradiënt, waarbij er typisch een hoge densiteit is in de kernpopulatie, en een lage densiteit aan het dispersiefront. Deze gradiënt in densiteit kan fenotypische plasticiteit induceren, resulterend in fenotypische divergentie tussen de kernpopulaties en populaties van het dispersiefront.

Gedurende areaalverbreiding verplaatst het dispersie front zich herhaaldelijk door middel van een reeks founder events, waarin slecht een aantal individuen het nieuwe habitat koloniseren, wat typerende gevolgen heeft voor de populatie structuur. Ten eerste leiden de herhaaldelijke founder events er toe dat individuen aan het dispersiefront een hogere mate van verwantschap vertonen aan elkaar, dan individuen van de kernpopulatie. Ten tweede leiden de lage densiteitscondities aan het dispersiefront er toe dat er op stochastische wijze genetische (en bijgevolg fenotypische) variatie verloren kan gaan. Bijgevolg wordt variatie aan het dispersiefront bepaald deels door deterministische processen (ruimtelijke selectie) en deels door stochastische processen (drift ten gevolge van de lage densiteit).

Traditioneel wordt areaalverbreiding vaak bestudeerd in de context van invasie voorvallen, waarbij een soort zich snel verbreidt in een nieuwe regio. Dit soort studies heeft echter vaak het nadeel dat er omgeving gebonden variatie is langs de routewaar de soort zich uitbreidde. Dit kan de interpretatie van de resultaten bemoeilijken, aangezien in bepaalde gevallen onderscheid tussen het effect van lokale adaptatie en ruimtelijke selectie niet mogelijk is. Daarom hebben we twee experimenten uitgedacht, gebruik makend van de bonenspintmijt (*Tetranychus urticae*) als model soort, waarbij er gewerkt wordt onder gecontroleerde omgeving variatie om bemoeilijking van de interpretatie te vermijden.

De experimentele opzet bestaat uit artificiële metapopulaties. Beide experimenten werden zo uitgedacht zodat evolutionaire processen (door ruimtelijke selectie) konden optreden in de helft van de opstellingen van elk experiment, terwijl in de andere helft evolutionaire processen verhinderd werden, terwijl densiteitscondities niet beïnvloed werden. In het eerste experiment werd evolutionair potentieel verhinderd door middel van een wekelijkse vervanging van alle volwassen vrouwtjes met individuen van een stock populatie. Op deze wijze werd ruimtelijke selectie verhinderd en de verwantschapsstructuur opgeheven, terwijl densiteitsgradiënt wel behouden werd. In het tweede experiment werd evolutionair potentieel verhinderd door het gebruik van inteelt lijnen, waar geen genetische variatie aanwezig was, waar selectie op kon werken. Weer werden de densiteitscondities niet beïnvloed. De combinatie van beide experimenten stelde ons in staat om een relatief volledige analyse te doen van alle factoren (ruimtelijke selectie, densiteitsgradiënt en verhoogd verwantschap) die fenotypische divergentie tussen kernpopulaties en populaties van het dispersiefront drijven.

In beide experimenten was er een significant snellere areaalverbreiding in de opstellingen waar evolutionair potentieel niet werd gelimiteerd ten opzicht van deze waar dit wel werd gedaan, wat aangeeft dat ruimtelijke selectie leidt tot een versnelling van areaalverbreiding. Het verschil was echter beduidend groter in het eerste experiment, wat impliceert dat, hoewel ruimtelijke selectie inderdaad een versnelling in areaalverbreiding drijft, het effect van verwantschap op snelheid van areaalverbreiding echter veel belangrijker is dan ruimtelijke selectie. Voorts leidde ruimtelijke selectie ook tot een verhoogde populatie groei aan het dispersiefront ten opzichte van de kernpopulatie in beide experimenten, wat overeenstemt met de theoretische verwachtingen omtrent selectie van een meer r-geselecteerde life history strategie aan het dispersiefront. Wanneer echter de individuele life history traits (gerelateerd aan fecunditeit, ontwikkelingstijd en overleving) bekeken werden, konden geen significante trends waargenomen worden. Mogelijks verloopt de adaptatie naar hogere populatiegroei volgens verschillende methoden in de verschillende metapopulaties volgens verschillende mechanismen, maar verdere analyses zijn nodig om dit met zekerheid te bepalen. Er werden geen verschillen in dispersie capaciteit waargenomen tussen populaties van de kern en van het dispersiefront. Er zijn verschillende mogelijke verklaringen waarom dit het geval was, waaronder evolutie van densiteitsafhankelijkheid van dispersie, en het niet geschikt zijn van de gebruikte methode of parameter om de verschillen waar te nemen.

Analyse van de drift effecten gaf aan dat er een zeer sterk en consequent initieel founder effect was, wat de variatie tussen metapopulaties sterk bepaalde. Ondanks dat dit minder consequent was dan het initieel founder effect, trad er toch regelmatig drift op gerelateerd aan de lage densiteit, wat er voor zorgde dat er op stochastische wijze fenotypische variatie verloren ging aan het dispersiefront.

Acknowledgements

Firstly, I would like to thank Dries Bonte, my supervisor for the opportunity to work in such an interesting project, and for the guidance and council he offered during the course of this project.

A great many thanks go to Katrien Van Petegem, my supervisor, who guided me through this project with an inspiring amount of enthusiasm, and whom I could always reach with the many questions I had concerning the work on this project, and who spent many hours aiding me with the analyses, and writing this dissertation.

Furthermore, I would like to thank Laure Hollez, Ruben Van De Walle and Mickey Sinnesael, for the work they have performed on this project, in the context of their own education, and who helped lighten the load of the experimental work.

The project often entailed a lot of practical work in order to maintain the experimental setups. At times, it would have been impossible to do all this work on my own, and therefore I would like to thank Mike Creutz and Matti Pissman, who spend many an hour aiding me on this project.

Lastly, I would like to thank the entire staff of the Terestrial Ecology unit, for the open and welcoming atmosphere, which made working there a more than pleasant endeavour. A special thanks go out to Frederik Mortier and Karen Bisschop, whose company and conversation made the long hours working in the laboratory feel a bit shorter, Maxim Dahirel, who took the time to look over and brainstorm over the results of this project, and Stefano Masier, who provided me with the script to analyse part of the data.

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Appendix 1: Statistical output and additional graphs

Population growth rate

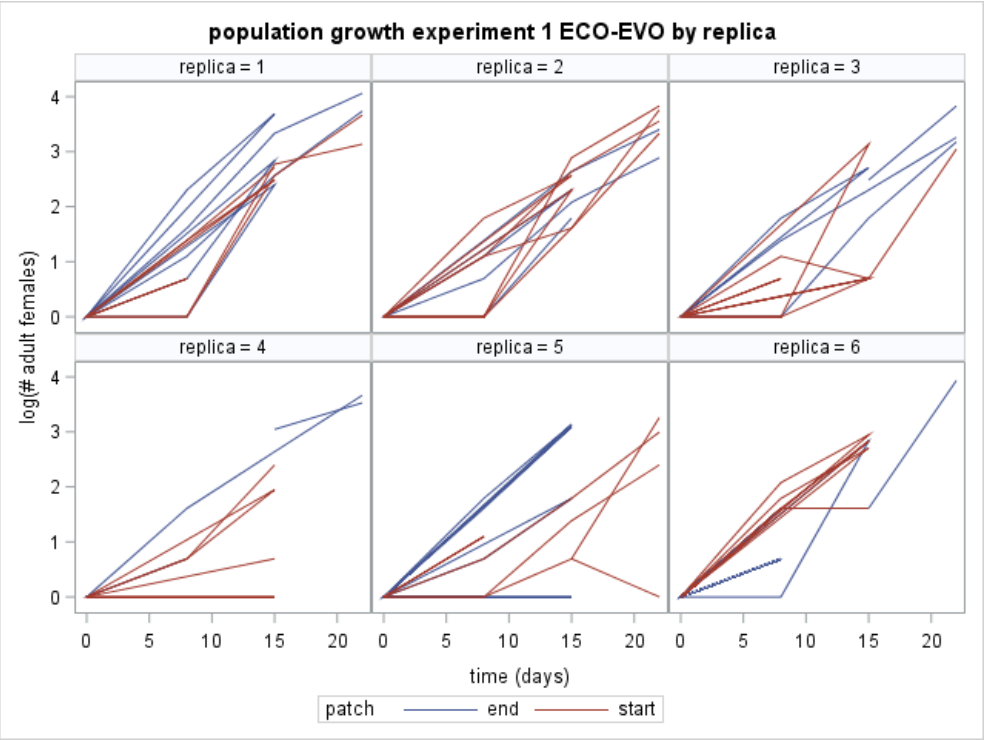
Experiment 1	ECO-EVO1	RFS
Start-end	Slope: **$F_{1,153}$=5.32; p=0.0225** Intercept: $F_{1,8.64}$=0.00; p=0.9823	Slope: $F_{1,117}$=0.31; p=0.5817 Intercept: $F_{1,10.3}$=0.69; p=0.4261
End-stock	Slope: $F_{1,99.2}$=3.16; p=0.0786 Intercept: **$F_{1,44.8}$=20.55; p<0.0001**	Slope: **$F_{1,136}$=15.21; p=0.0002** Intercept: $F_{1,113}$=0.48; p=0.4893
Start-stock	Slope: **$F_{1,108}$=15.85; p=0.0001** Intercept: $F_{1,3.85}$=1.57; p=0.2815	Slope: **$F_{1,132}$=5.08; p=0.0259** Intercept: $F_{1,1.5}$=0.05; p=0.8566

Appendix 1. 1: Statistical output for population growth rate for experiment 1

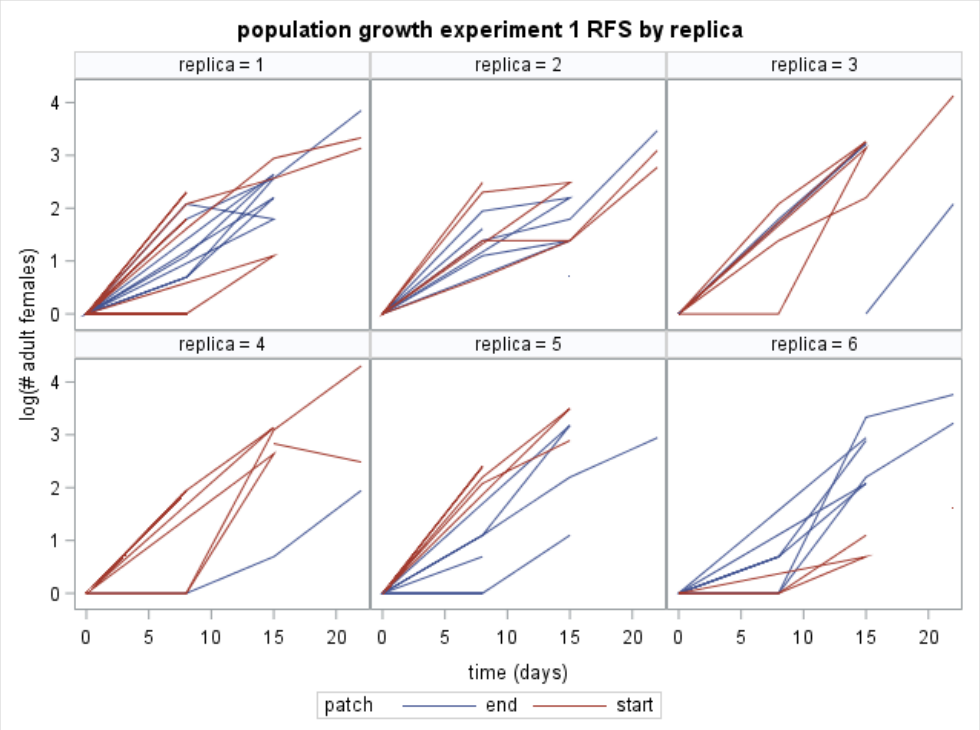
Experiment 2	ECO-EVO2	ECO
Start-end	Slope: **$F_{1,235}$=6.46; p=0.0117** Intercept: $F_{1,9.45}$=1.12; p=0.3159	Slope: $F_{1,278}$=0.51; p=0.4747 Intercept: $F_{1,20}$=0.25; p=0.6256
End-stock	Slope: **$F_{1,141}$=6.02; p=0.0154** Intercept: $F_{1,12.3}$=0.51; p=0.4875	Slope: $F_{1,216}$=1.47; p=0.2274 Intercept: $F_{1,22.4}$=0.10; p=0.7504
Start-stock	Slope: $F_{1,56.2}$=1.49; p=0.2272 Intercept: $F_{1,7.88}$=0.01; p=0.9085	Slope: $F_{1,215}$=0.80; p=0.3712 Intercept: $F_{1,13.6}$=0.00; p=0.9933

Appendix 1. 2: Statistical output for population growth rate for experiment 2

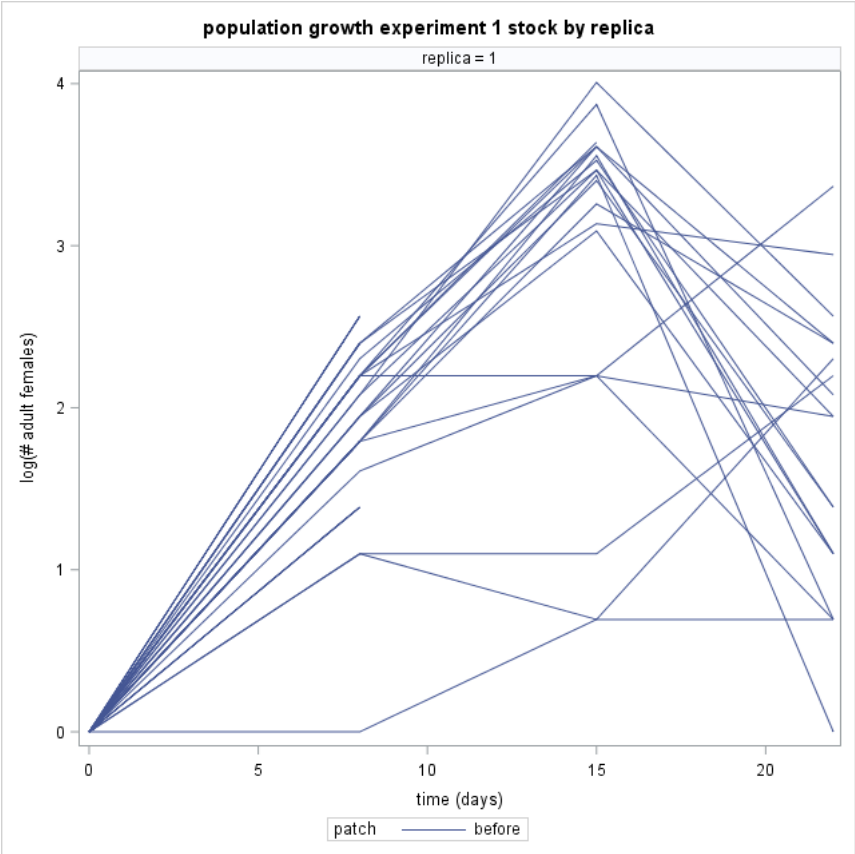
Whereas the differences between the core (start) and dispersal front (end) populations is clear across both experiments, no such clear trend can be found for the comparisons with the stock population. In the first experiment, adaptation appears to have occurred in the core, but in the second experiment, only stock and dispersal front differ significantly in the evolutionary unconstrained setups. For the evolutionary constrained setups, results differ between experiments as well. Whereas both core and dispersal front populations differ in population growth rate from the stock (but not from each other), no differences can be observed for the second experiment.



Appendix 1. 3: Population growth rate of ECO-EVO1 treatment of experiment 1 by replica



Appendix 1. 4: Population growth rate of RFS treatment of experiment 1 by replica



Appendix 1. 5: Population growth rate of stock of experiment 1 by replica

Egg survival

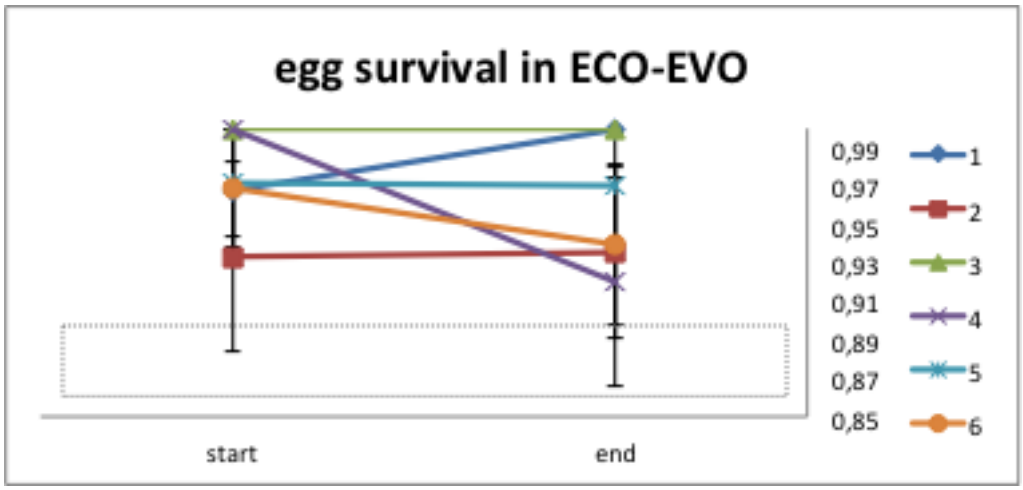
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,366.4}$=0.18; p=0.6718	**$F_{1,6.486}$=5.84; p=0.0490**
End-stock	$F_{1,1.604}$=1.88; p=0.3313	$F_{1,3.586}$=0.02; p=0.9028
Start-stock	$F_{1,8.959}$=4.01; p=0.0763	$F_{1,10.48}$ =4.75; p=0.0532

Appendix 1. 6: Statistical output for egg survival of experiment 1

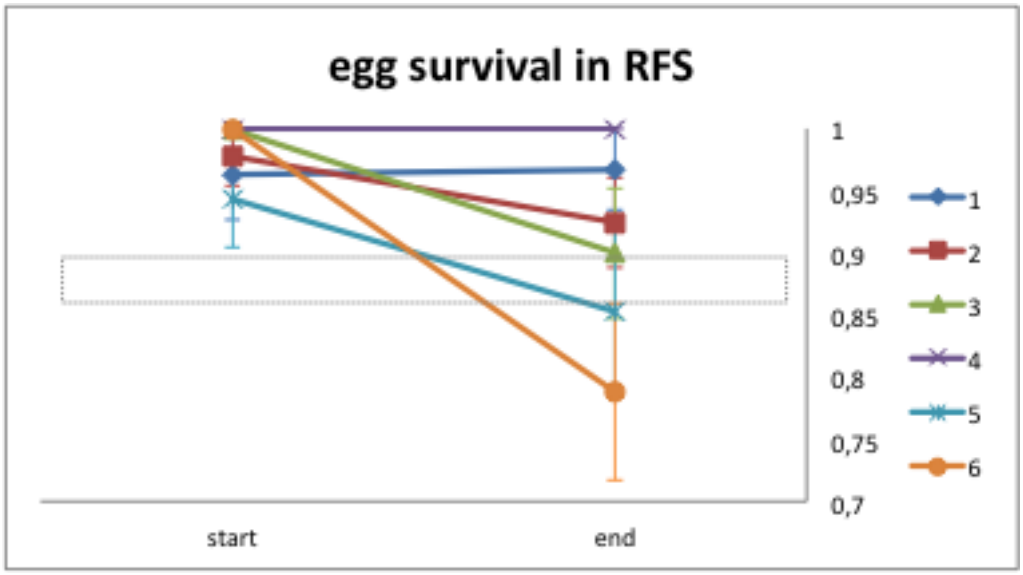
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,6.685}$=0.74; p=0.4205	$F_{1,8.774}$=0.17; p=0.6869
End-stock	$F_{1,6.279}$=1.27; p=0.3002	$F_{1,3.867}$=0.06; p=0.8251
Start-stock	$F_{1,4.215}$=1.25; p=0.3227	$F_{1,4.577}$=0.02; p=0.8835

Appendix 1. 7: Statistical output for egg survival of experiment 2

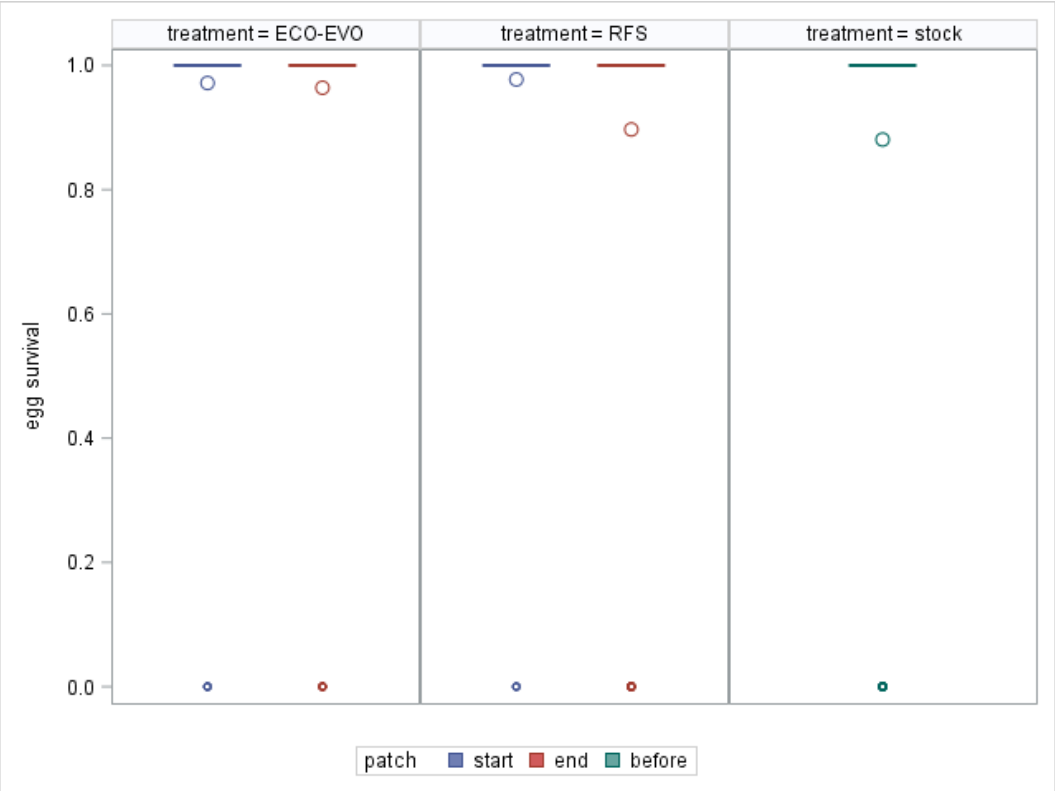
No significant differences between either core or dispersal front populations with the stock population, in terms of egg survival could be detected.



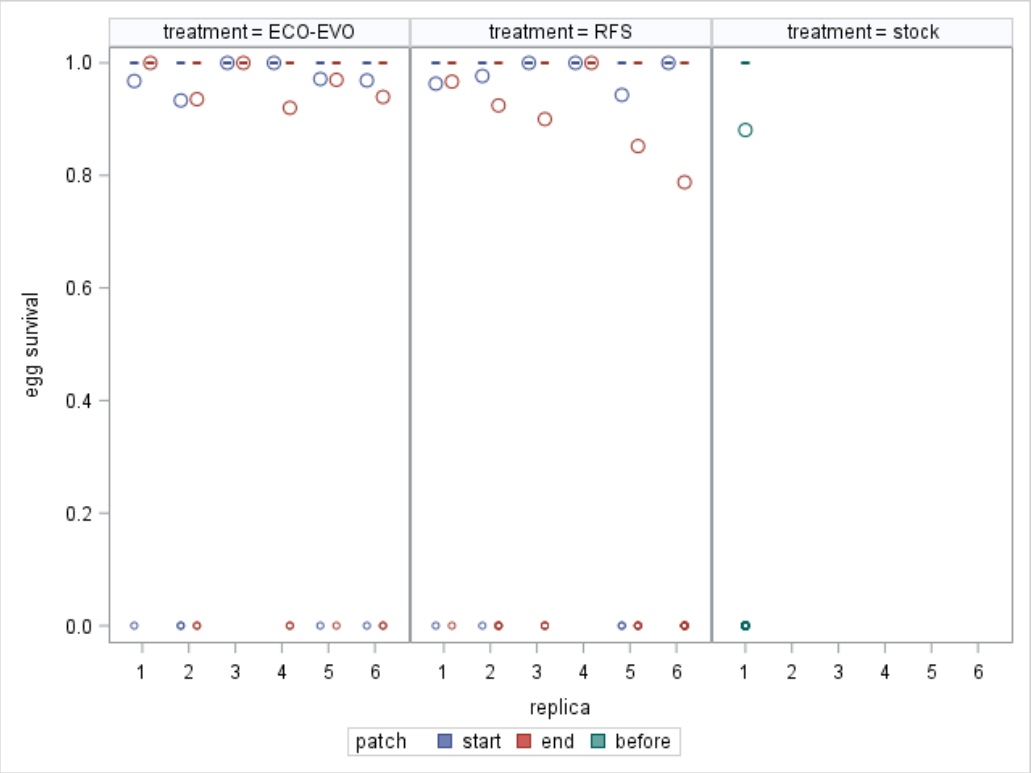
Appendix 1. 8: Effect sizes by replica of egg survival for ECO-EVO1 treatment of experiment 1



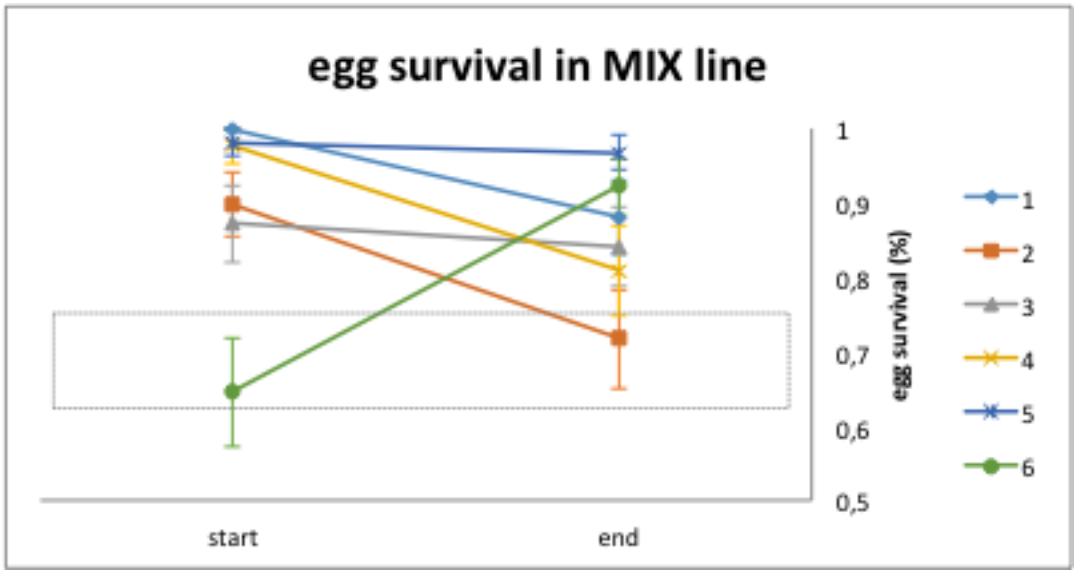
Appendix 1. 9: Effect sizes by replica of egg survival for RFS treatment of experiment 1



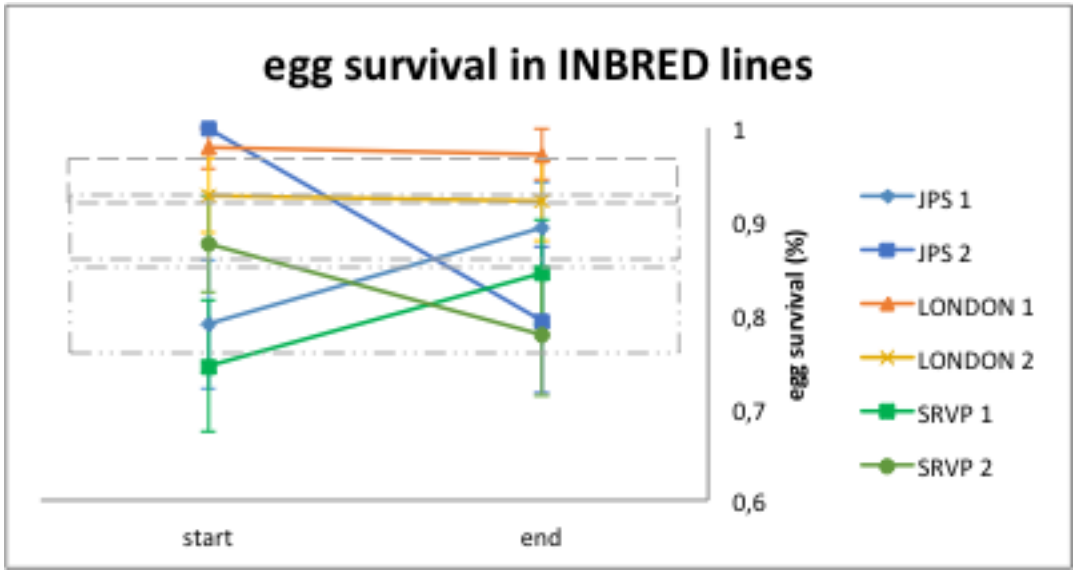
Appendix 1. 10: Egg survival of experiment 1 by treatment and patch



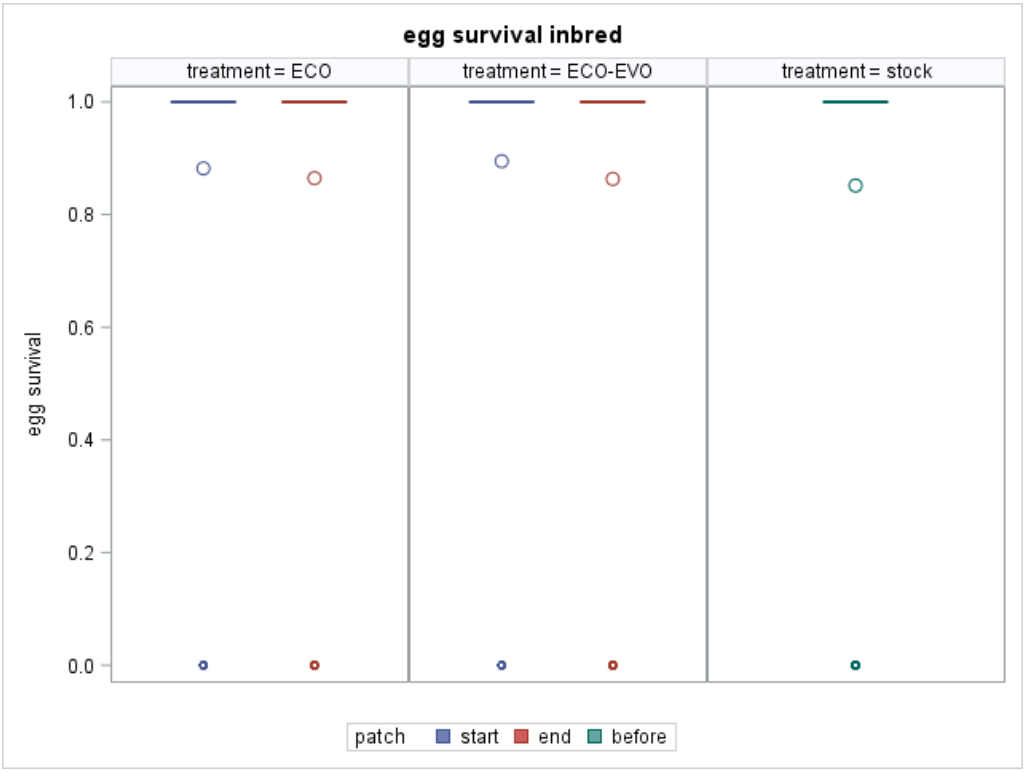
Appendix 1. 11: Egg survival of experiment 1 by replica and patch



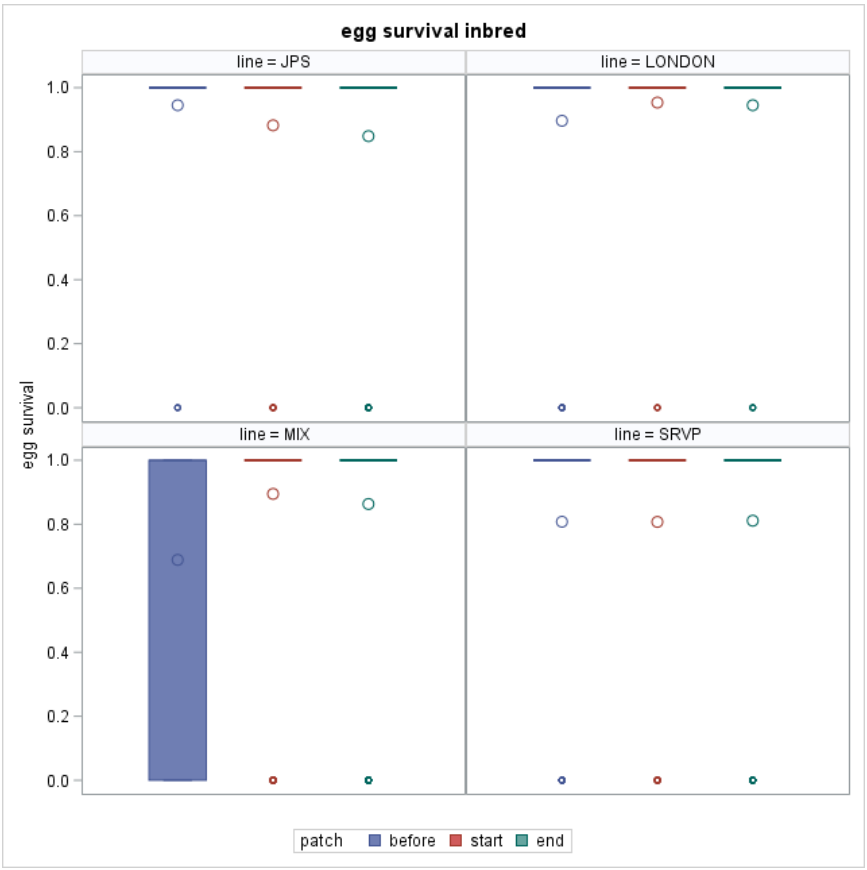
Appendix 1. 12: Effect sizes by replica of egg survival for the ECO-EVO2 treatment of experiment 2



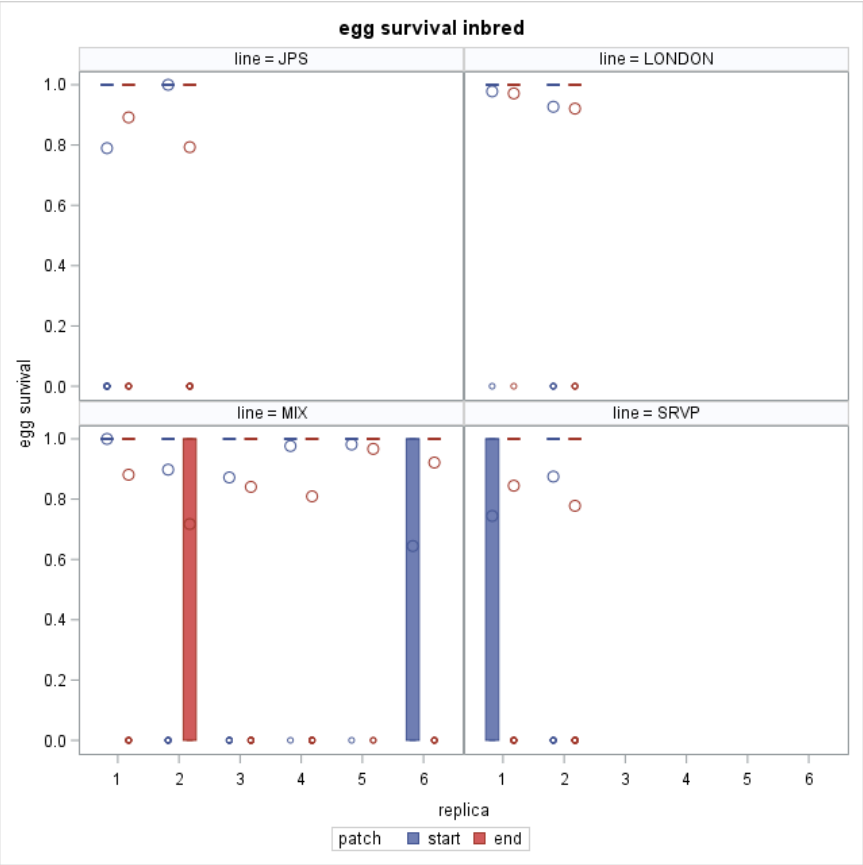
Appendix 1. 13: Effect sizes by replica of egg survival for the ECO treatment of experiment 2



Appendix 1. 14: Egg survival by treatment and patch for experiment 2



Appendix 1. 15: Egg survival by line and patch for experiment 2



Appendix 1. 16: Egg survival by replica and patch for experiment 2

Juvenile survival

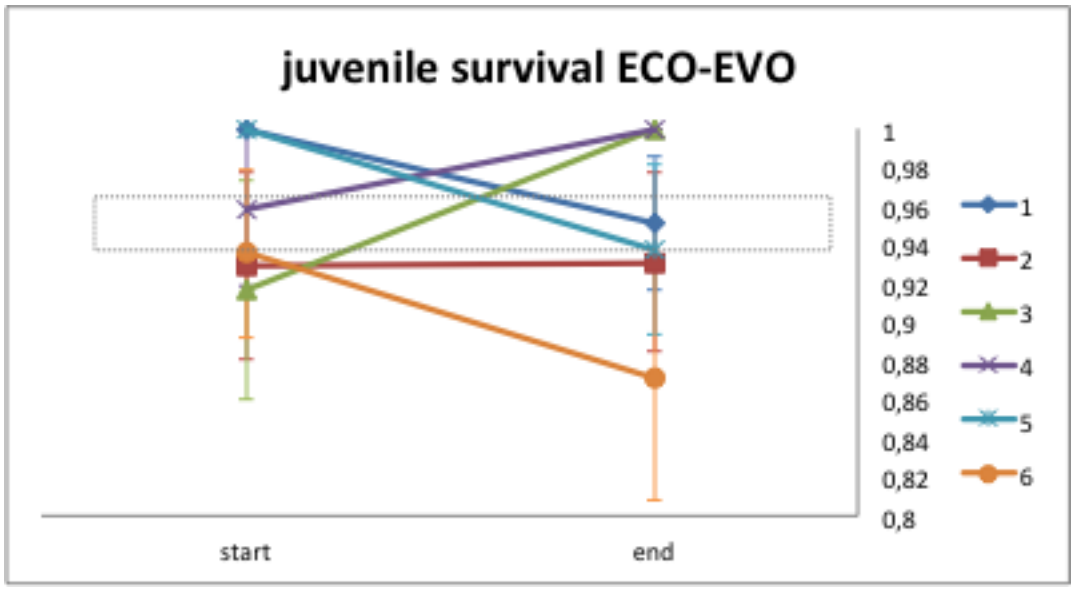
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,1.746}$=0.10; p=0.7826	$F_{1,4.246}$=0.02; p=0.9020
End-stock	$F_{1,4.295}$=0.02; p=0.9010	$F_{1,3.407}$=0.00; p=0.9827
Start-stock	$F_{1,5.43}$=0.18; p=0.6876	$F_{1,2.974}$=0.00; p=0.9749

Appendix 1. 17: Statistical output for juvenile survival of experiment 1

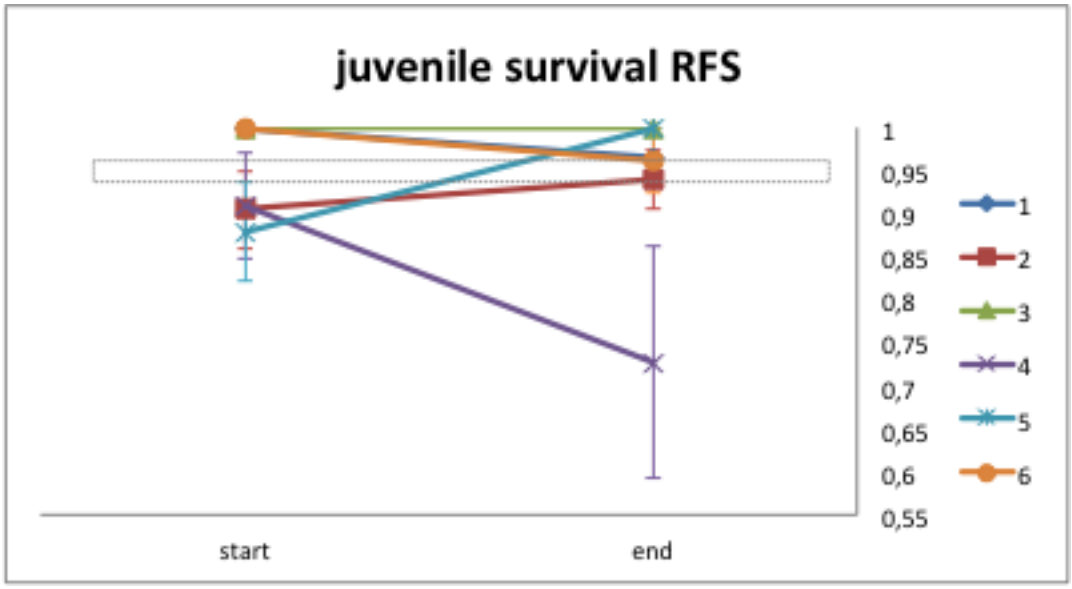
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,476.9}$=2.39; p=0.1226	**$F_{1,5.284}$=10.77; p=0.0202**
End-stock	$F_{1,3.266}$=0.61 p=0.4872	$F_{1,10.29}$=2.40; p=0.1515
Start-stock	$F_{1,2.566}$=1.35; p=0.3422	$F_{1,7.724}$=3.98; p=0.0825

Appendix 1. 18: Statistical output for juvenile survival of experiment 2

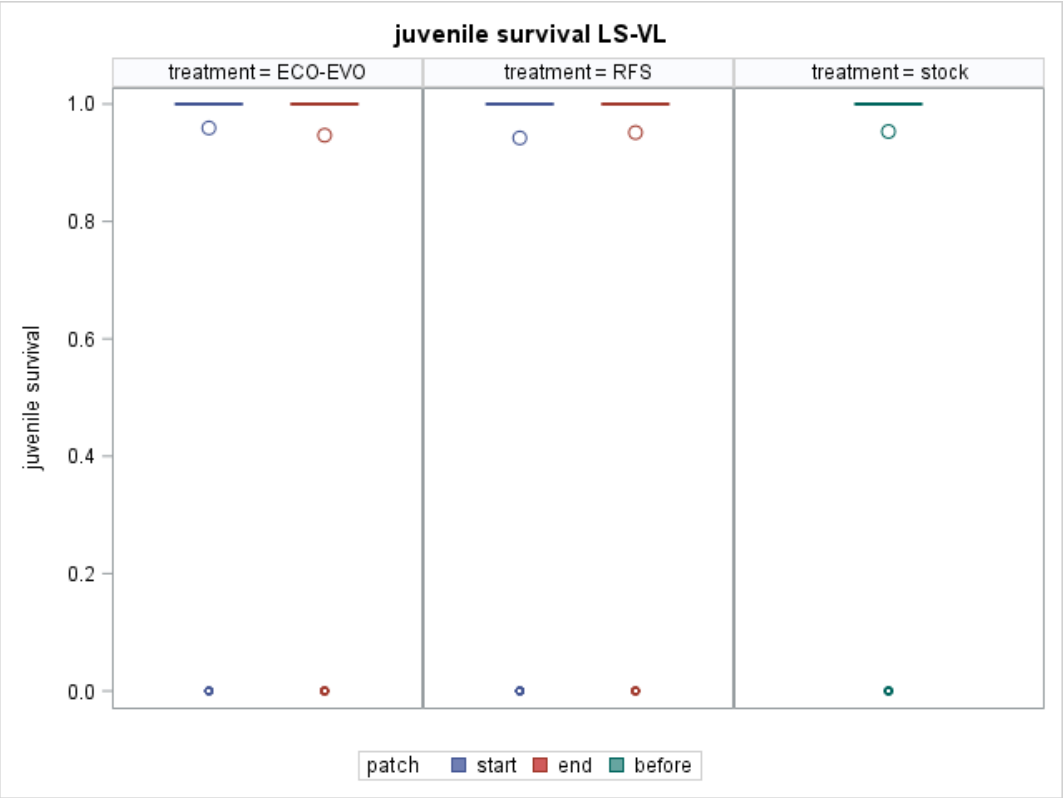
No significant differences in juvenile survival between any of the treatments and the stock population could be detected.



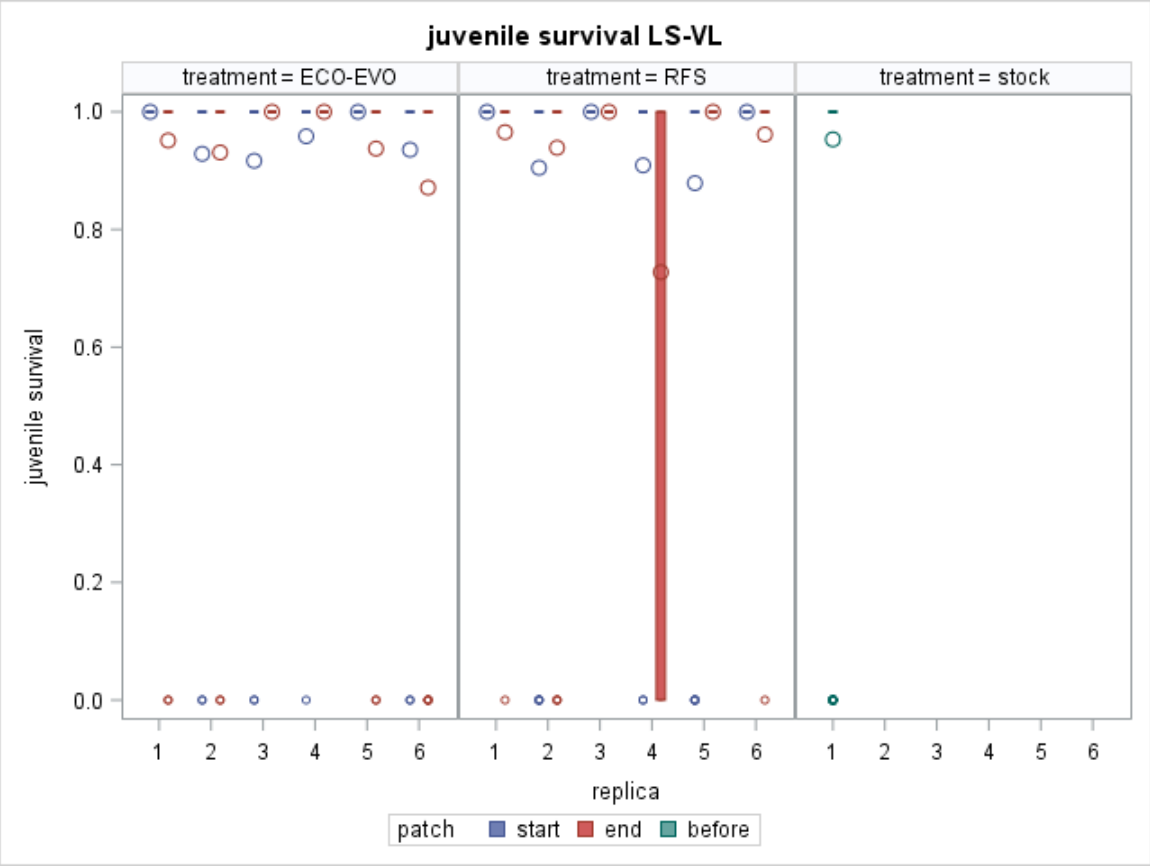
Appendix 1. 19: Effect sizes by replica of juvenile survival for the ECO-EVO1 treatment of experiment 1



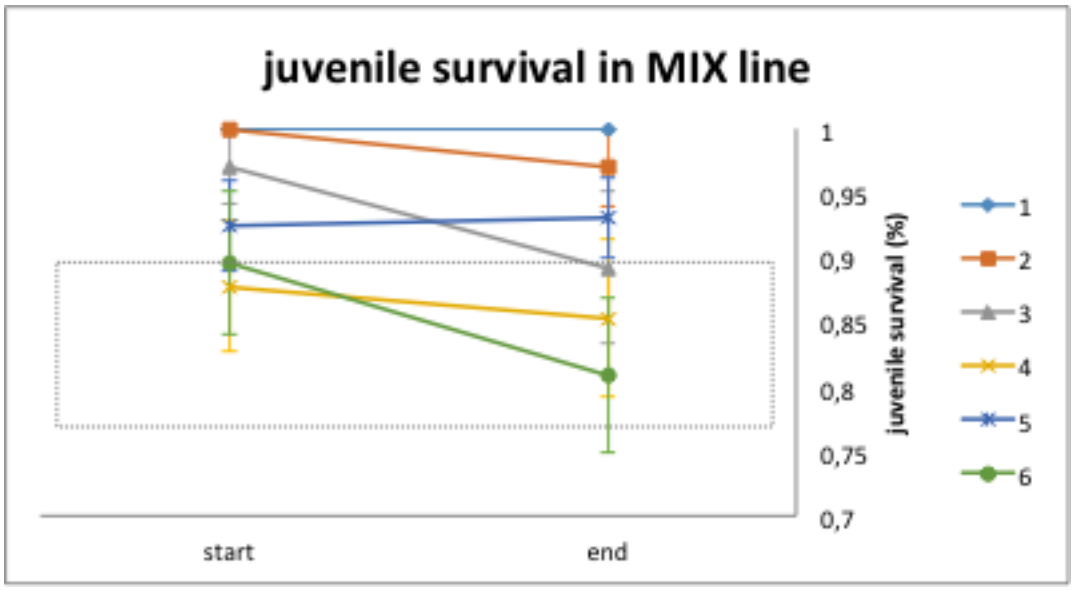
Appendix 1. 20: Effect sizes by replica of juvenile survival for the ECO treatment of experiment 1



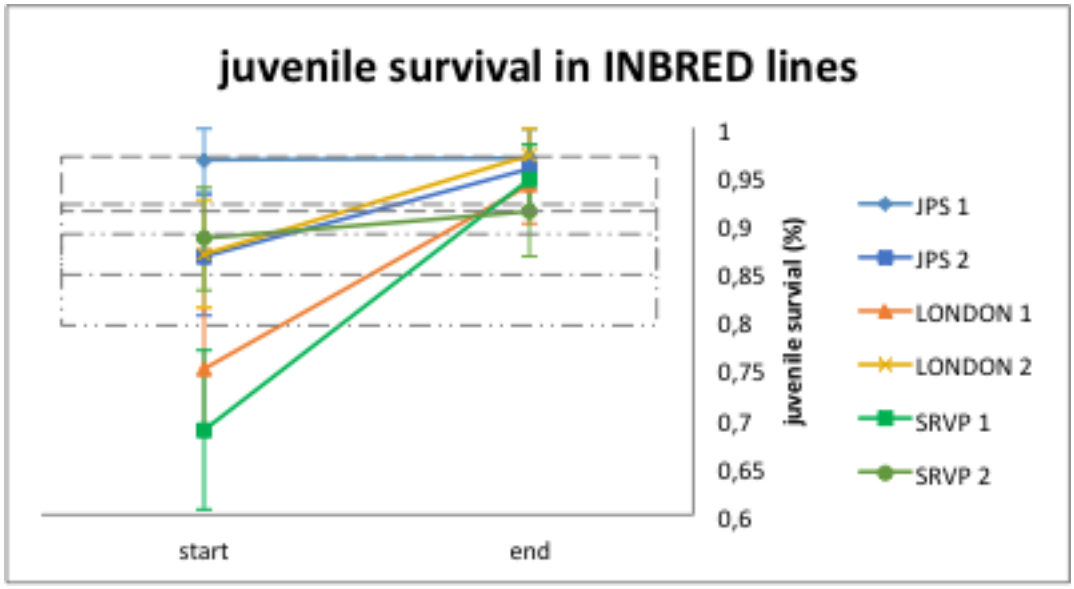
Appendix 1. 21: Juvenile survival by treatment and patch for experiment 1



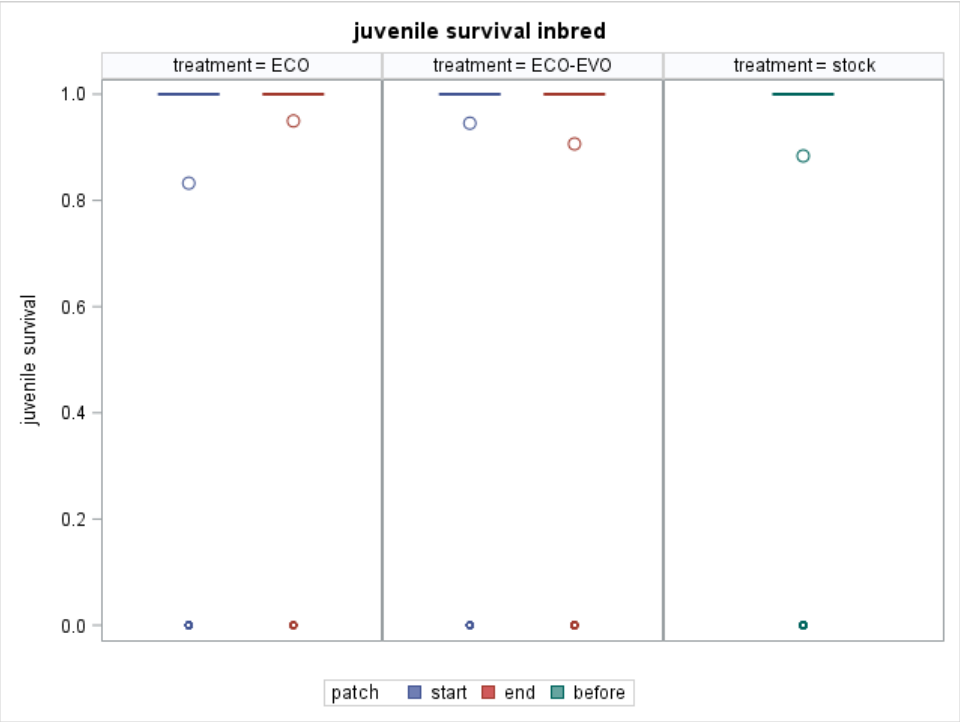
Appendix 1. 22: Juvenile survival by replica and patch for experiment 1



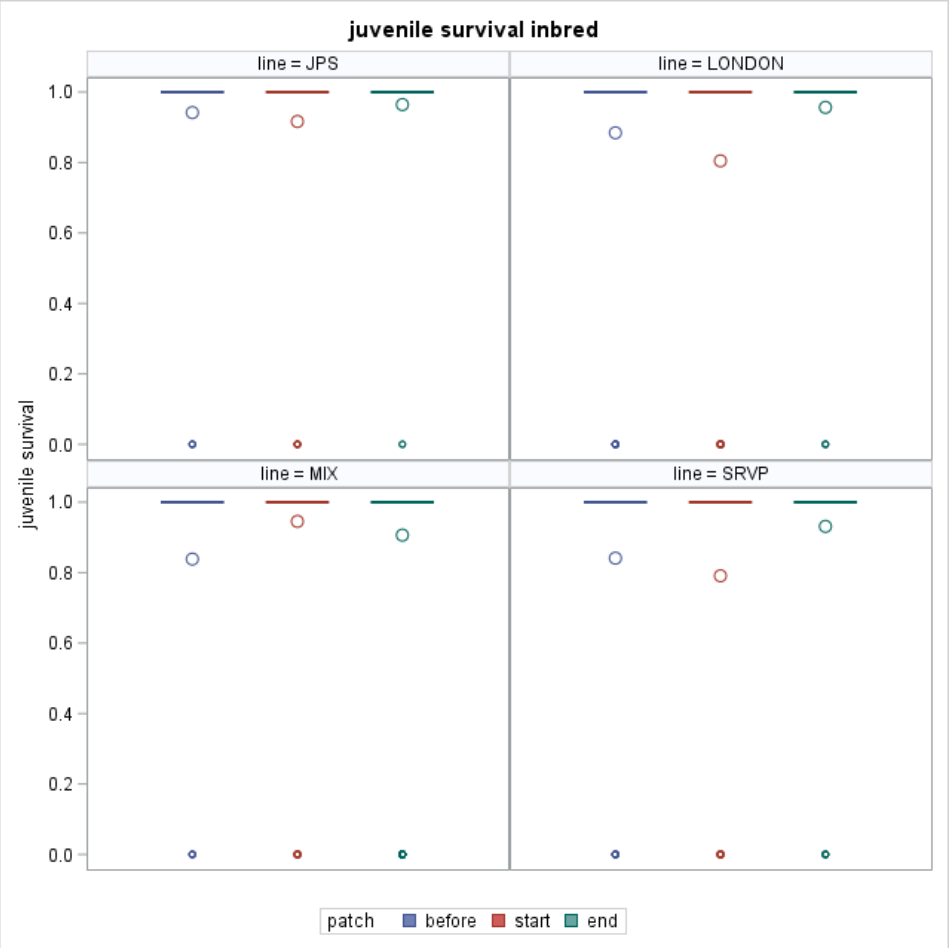
Appendix 1. 23: Effect sizes by replica of juvenile survival for the ECO-EVO2 treatment of experiment 2



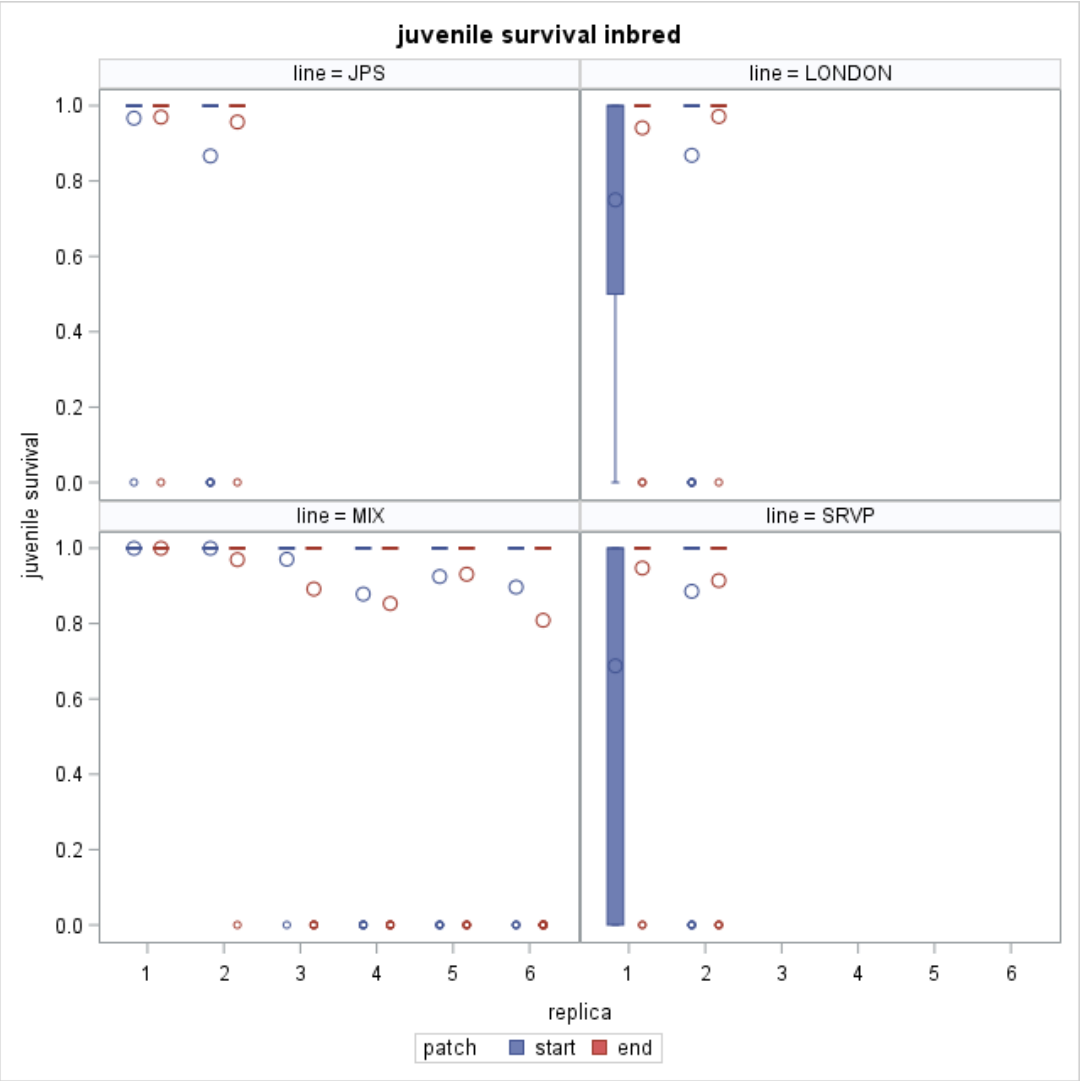
Appendix 1. 24: Effect sizes by replica of juvenile survival for the ECO-EVO2 treatment of experiment 2



Appendix 1. 25: Juvenile survival by treatment and patch for experiment 2



Appendix 1. 26: Juvenile survival by line and patch for experiment 2



Appendix 1. 27: Juvenile survival by replica and patch for experiment 2

Development time

Experiment 1 females	ECO-EVO1	RFS
Start-end	$F_{1,2.25}$=0.66; p=0.4937	$F_{1,2.37}$=0.06; p=0.8328
End-stock	$F_{1,3.9}$=0.20; p=0.6782	$F_{1,2.38}$=0.56; p=0.5198
Start-stock	$F_{1,5.45}$=0.55; p=0.4900	$F_{1,2.78}$=0.78; p=0.4460

Appendix 1. 28: Statistical output for female development time of experiment 1

Experiment 1 males	ECO-EVO1	RFS
Start-end	$F_{1,3.72}$=0.96 p=0.3860	$F_{1,142}$=0.09; p=0.7611
End-stock	$F_{1,7.67}$=0.91; p=0.3695	$F_{1,6.16}$=0.74; p=0.4217
Start-stock	$F_{1,5.17}$=1.06; p=0.3497	$F_{1,7.35}$=1.22; p=0.3041

Appendix 1. 29: Statistical output for male development time of experiment 1

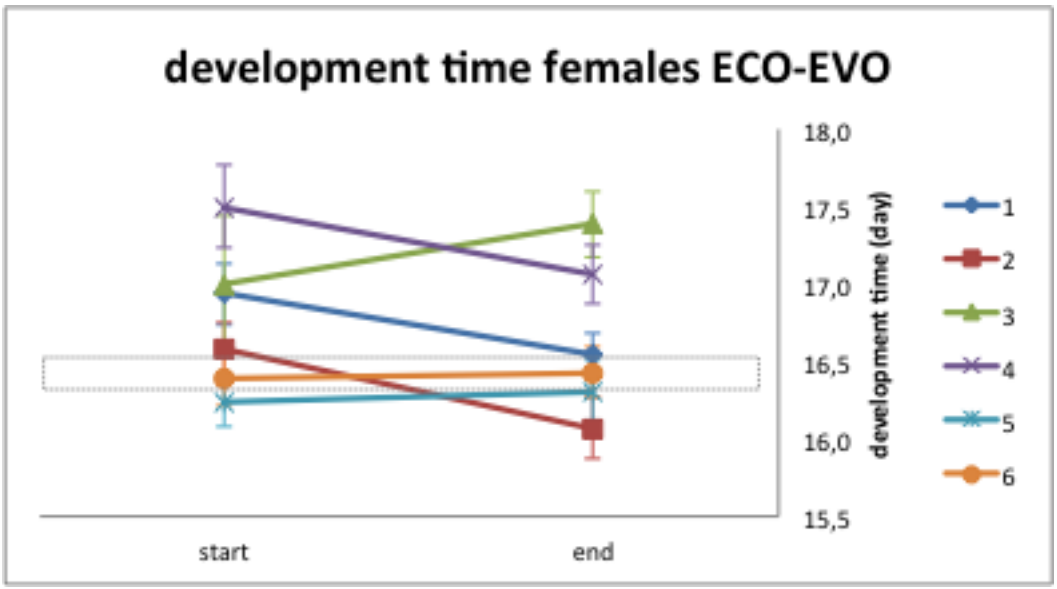
Experiment 2 females	ECO-EVO2	ECO
Start-end	$F_{1,7.69}$=0.02; p=0.8981	$F_{1,5}$=0.96; p=0.3719
End-stock	$F_{1,9.16}$=0.04; p=0.8523	$F_{1,8.52}$=0.36; p=0.5639
Start-stock	$F_{1,6.68}$=0.11; p=0.7535	$F_{1,13}$=0.58; p=0.4585

Appendix 1. 30: Statistical output for female development time of experiment 2

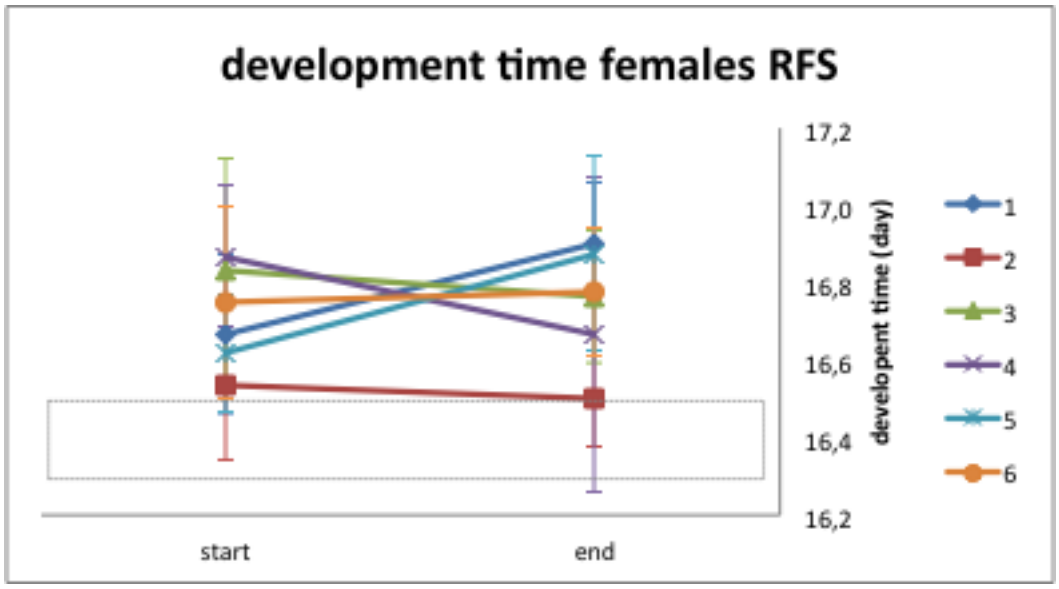
Experiment 2 males	ECO-EVO2	ECO
Start-end	$F_{1,9.64}$=0.18; p=0.6845	$F_{1,6.28}$=0.11; p=0.7536
End-stock	$F_{1,8.64}$=4.52; p=0.0636	$F_{1,2.61}$=2.20; p=0.2473
Start-stock	**$F_{1,8.13}$=7.51; p=0.0250**	$F_{1,3.74}$=2.82; p=0.1734

Appendix 1. 31: Statistical output for male development time of experiment 2

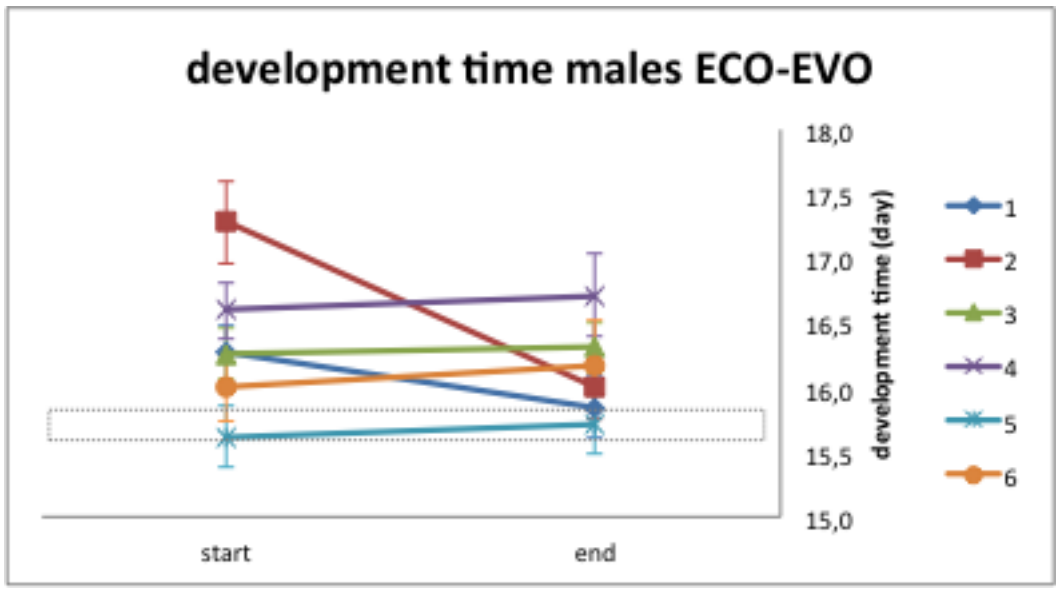
Only male development time of individuals from the core population of the ECO-EVO2 treatment from experiment 2 differs from the stock population, but no clear trends can be discerned whether adaptation occurs at the core or at the dispersal front.



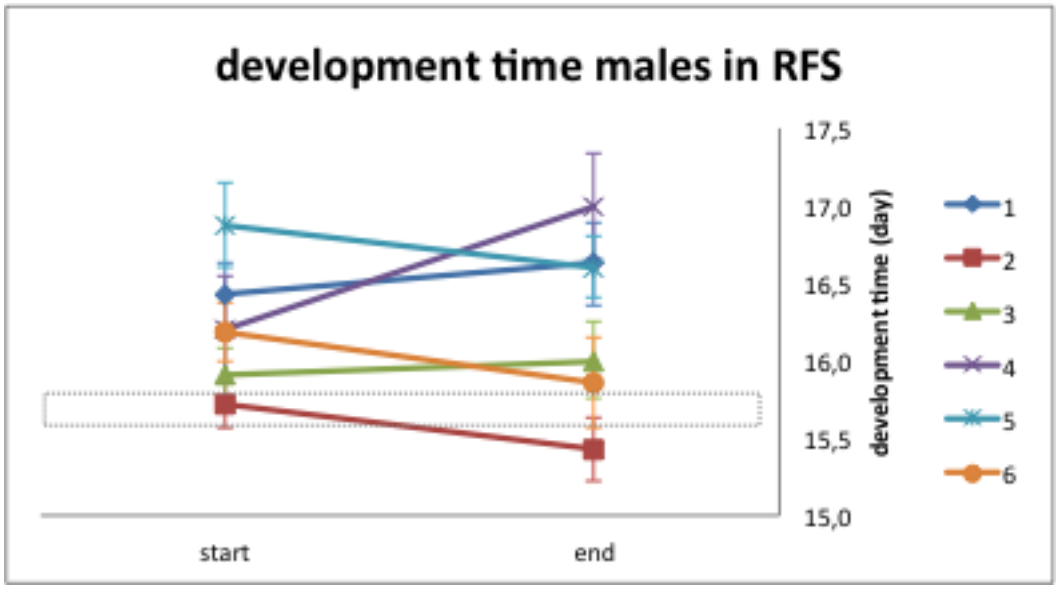
Appendix 1. 32: Effect sizes by replica for female development time of the ECO-EVO1 treatment of experiment 1



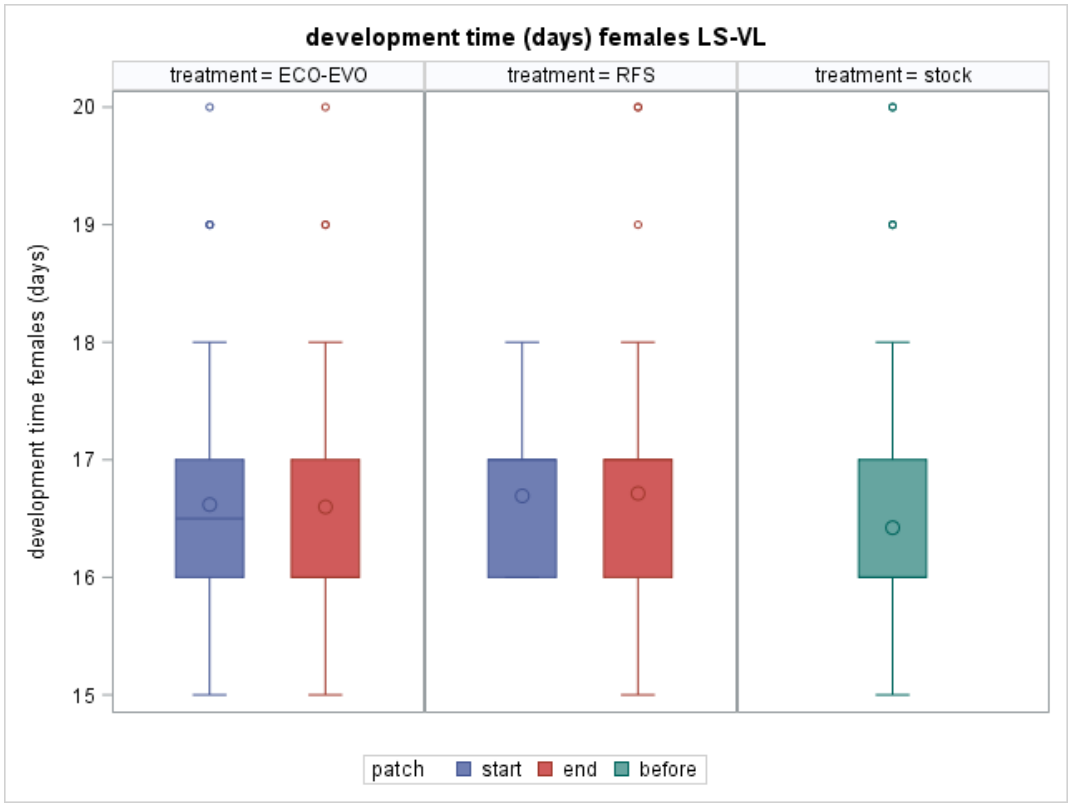
Appendix 1. 33: Effect sizes by replica for female development time of the RFS treatment of experiment 1



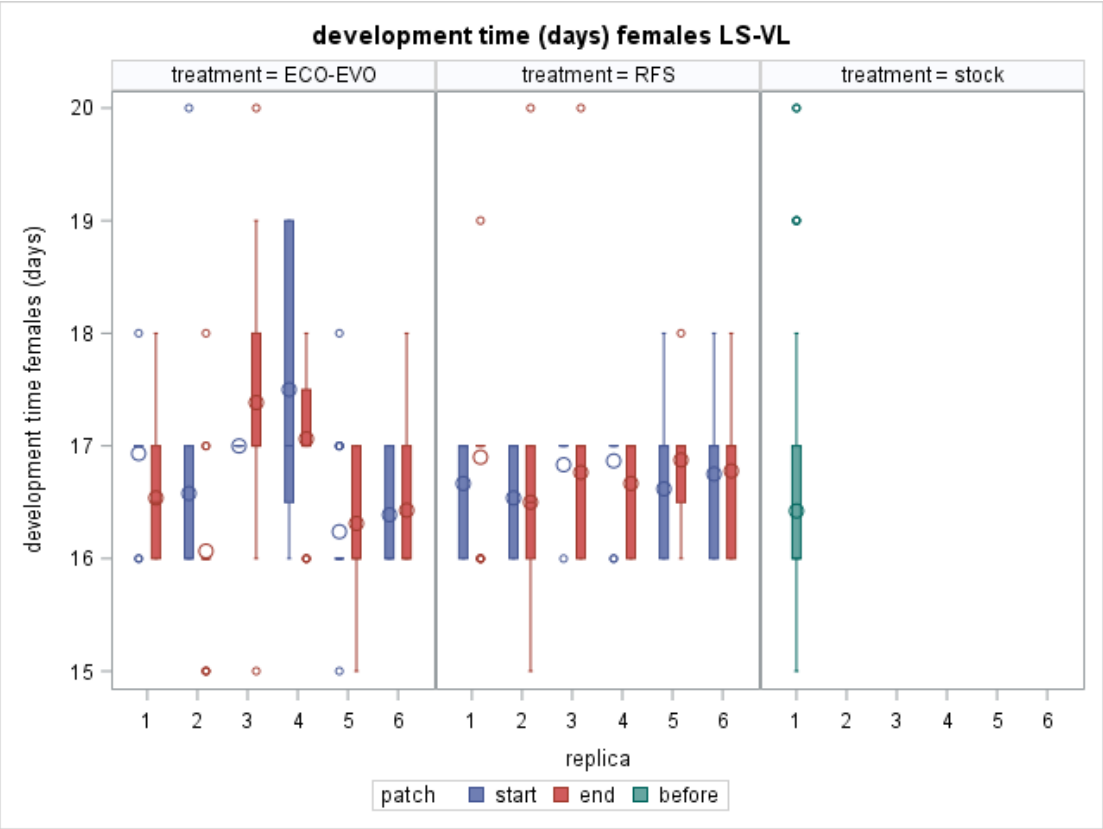
Appendix 1. 34: Effect sizes by replica for male development time of the ECO-EVO1 treatment of experiment 1



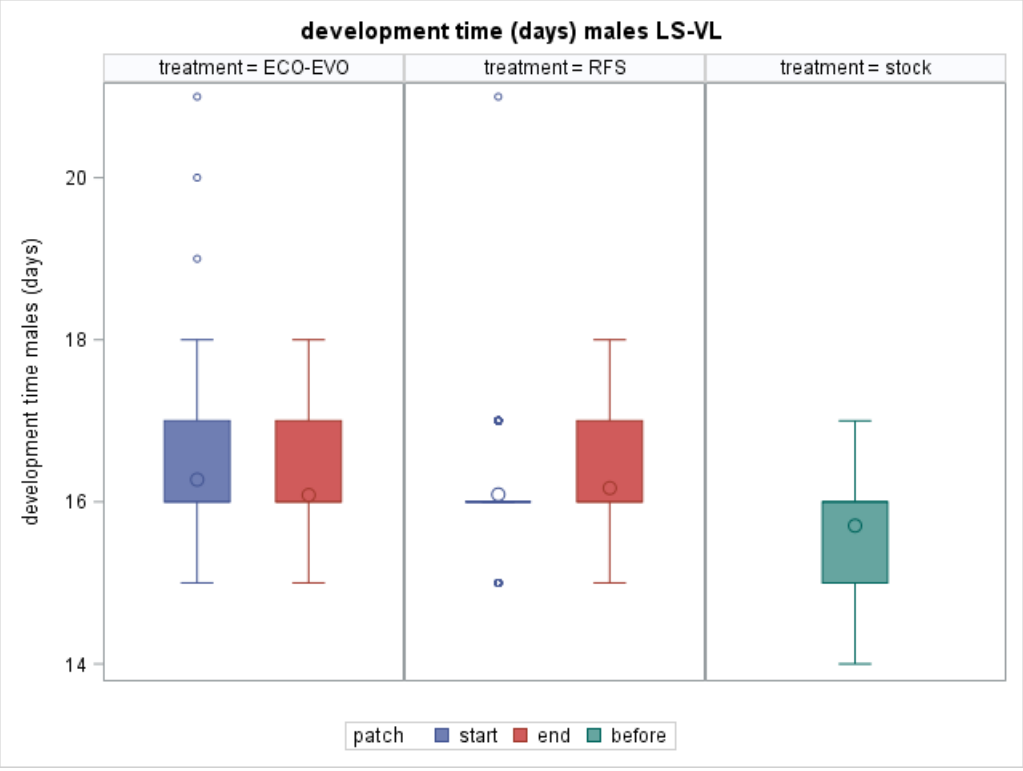
Appendix 1. 35: Effect sizes by replica for male development time of the RFS treatment of experiment



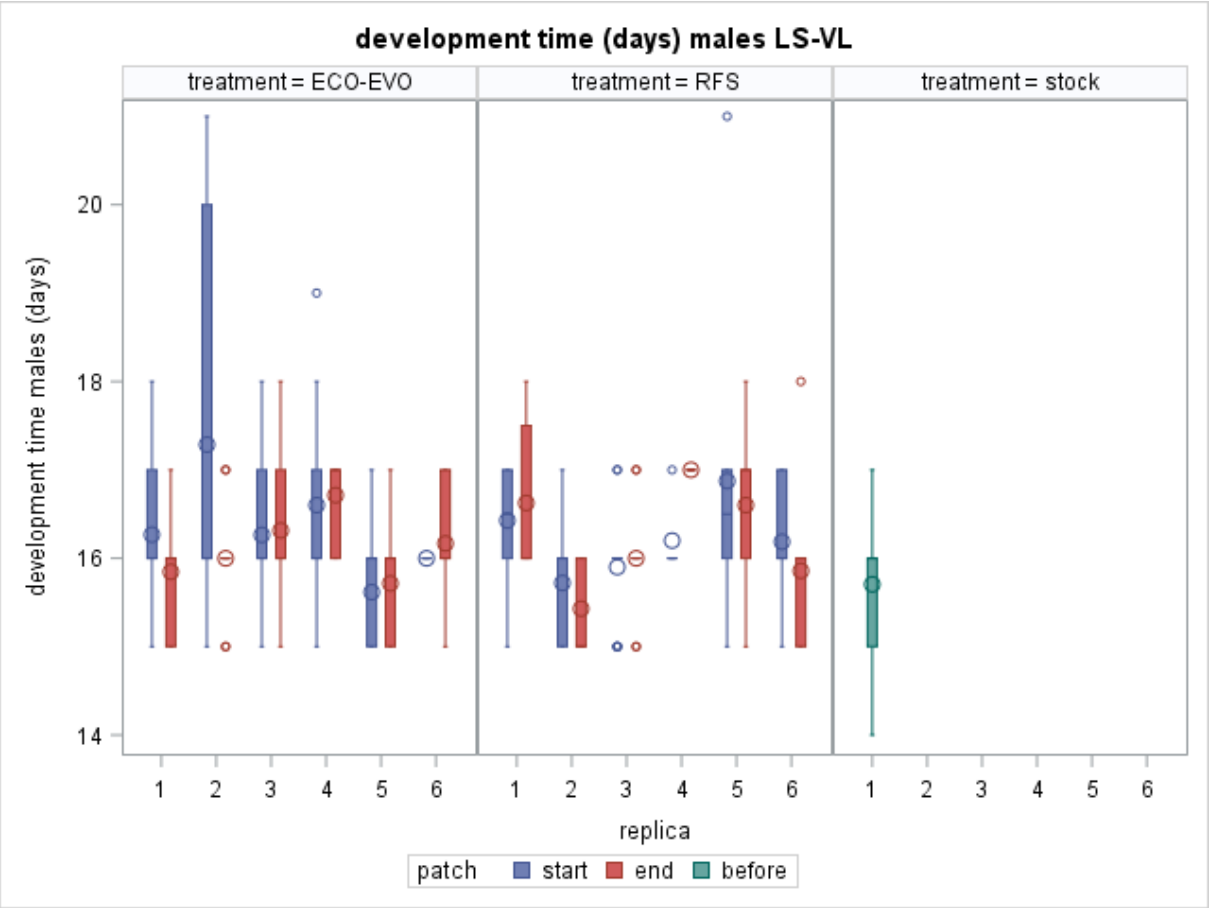
Appendix 1. 36: Female developmenty time by treatment and patch for experiment 1



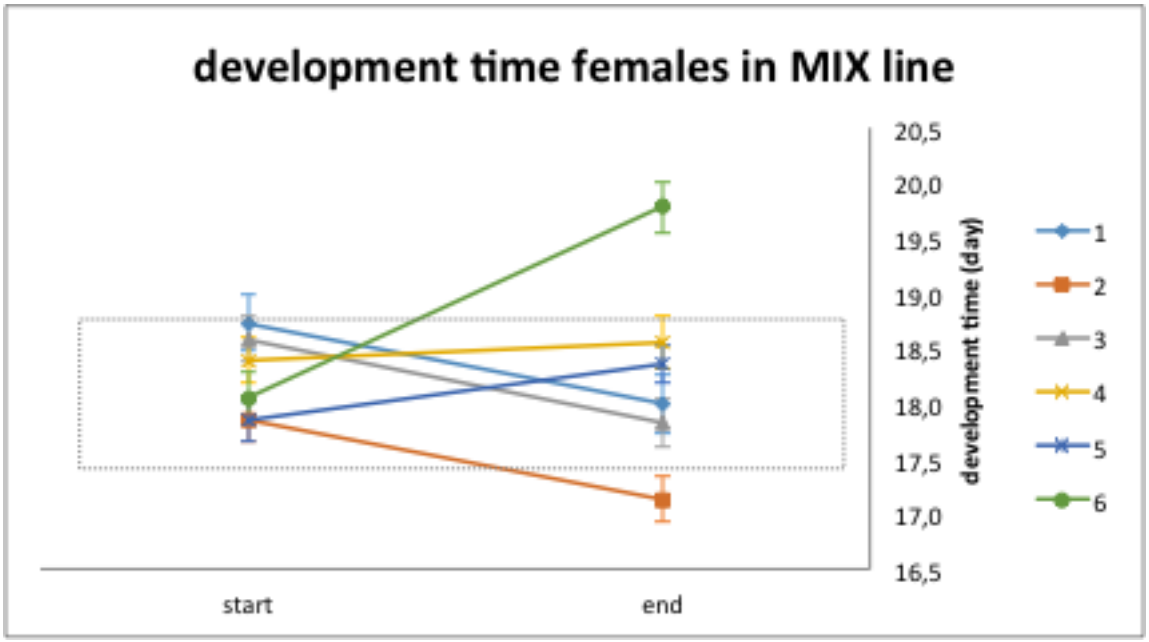
Appendix 1. 37: Female development time by replica and patch for experiment 1



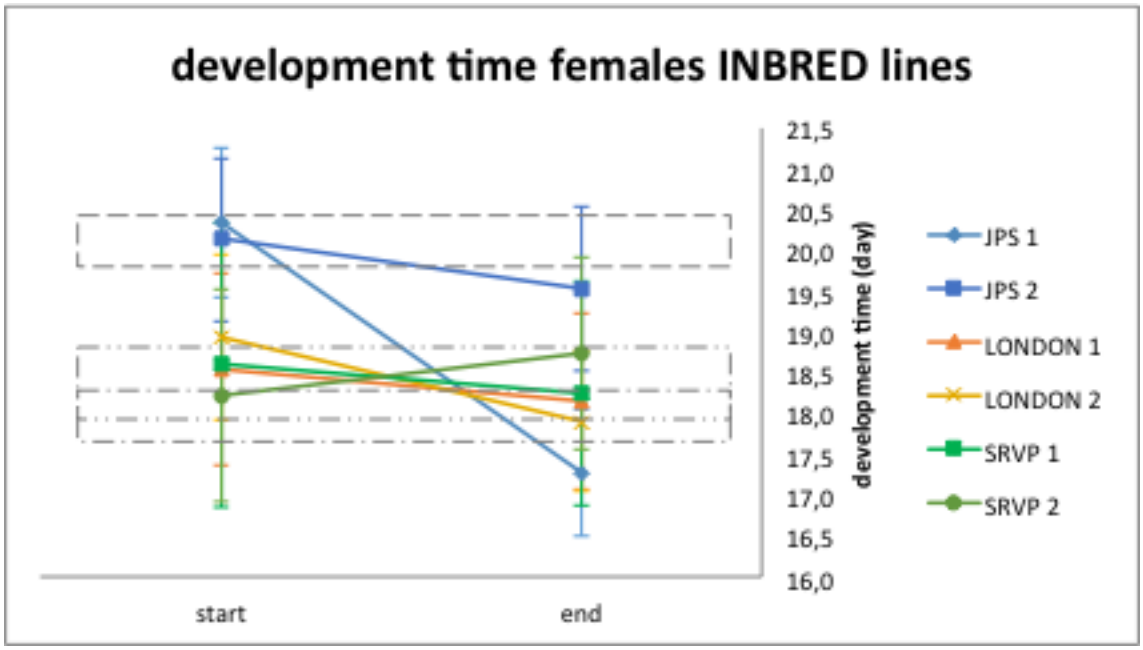
Appendix 1. 38: Male development time by treatment and patch for experiment 1



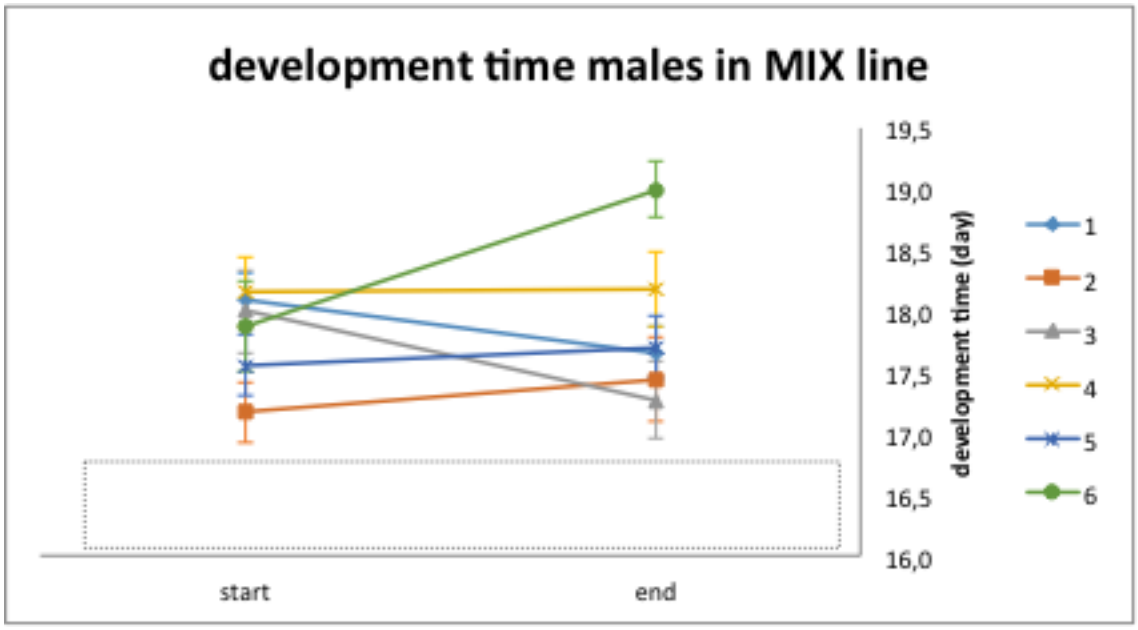
Appendix 1. 39: Male development time by replica and patch for experiment 1



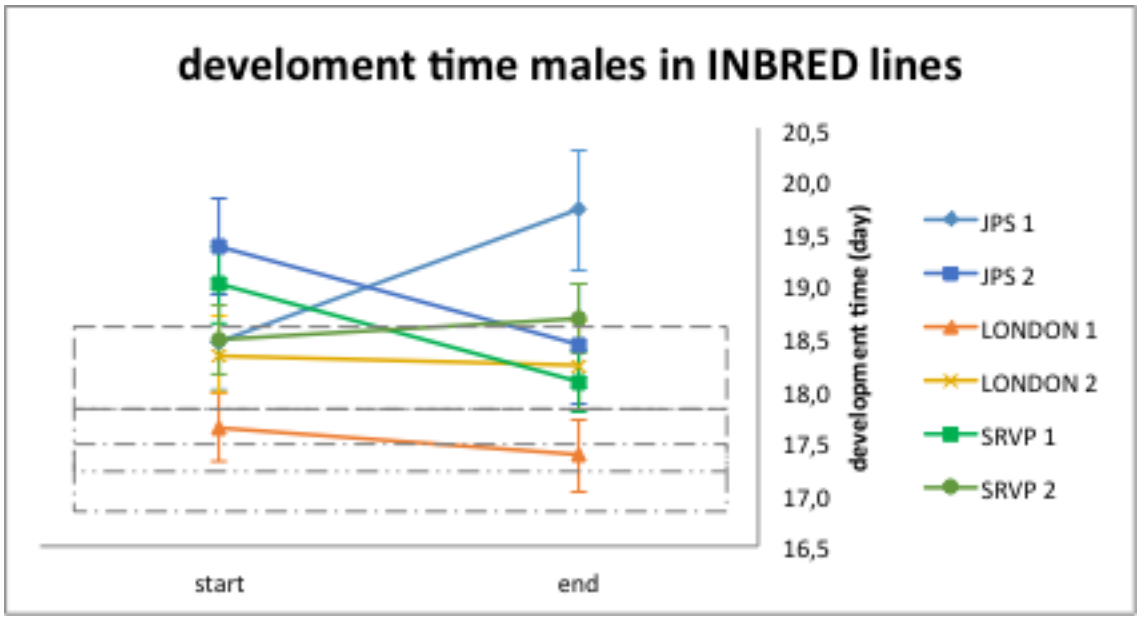
Appendix 1. 40: Effect sizes by replica for female development time of the ECO-EVO2 treatment of experiment 2



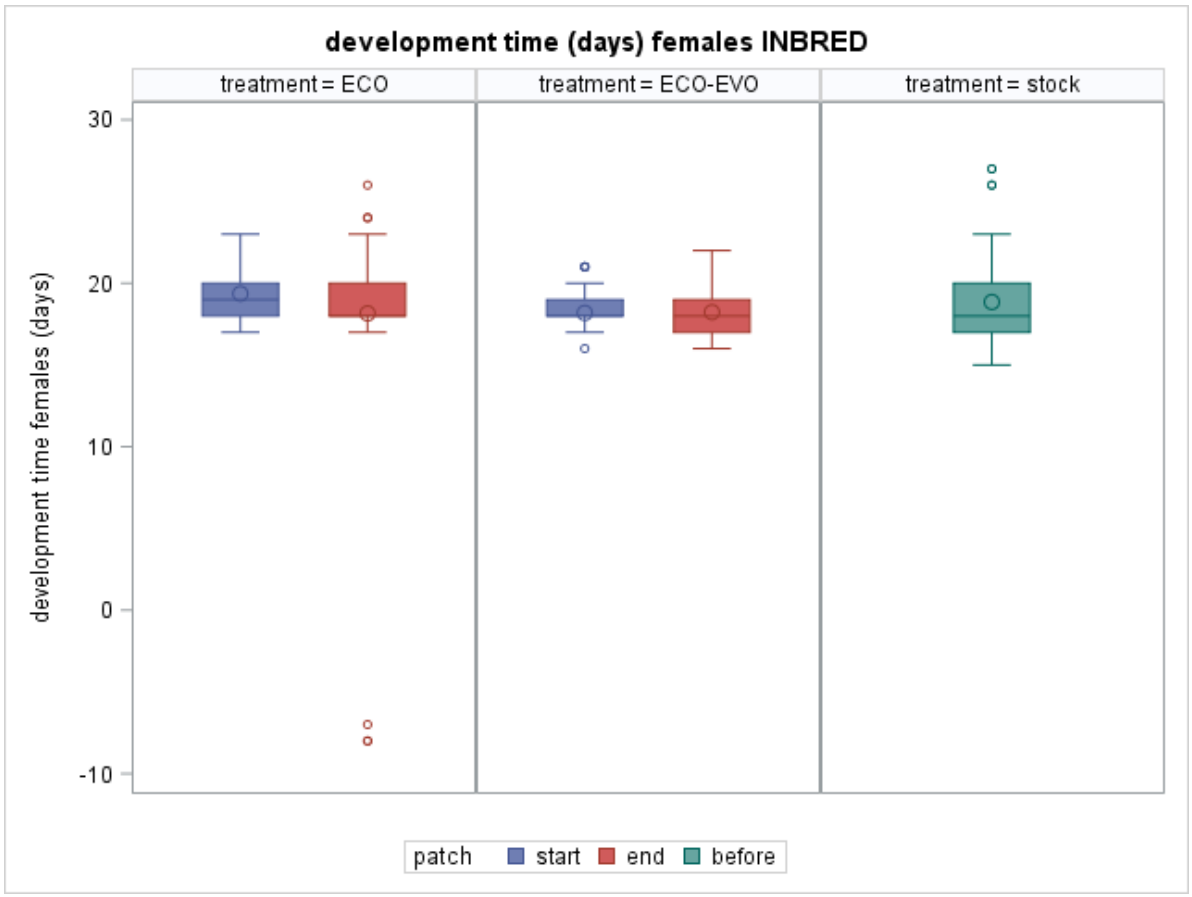
Appendix 1. 41: Effect sizes by replica for female development time of the ECO treatment of experiment 2



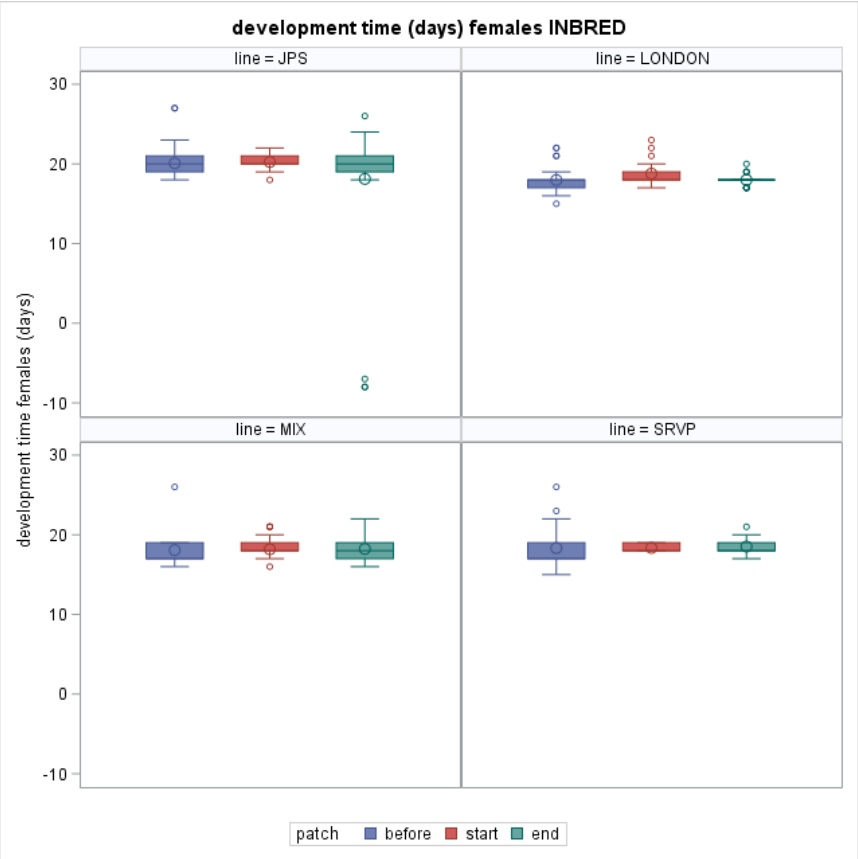
Appendix 1. 42: Effect sizes by replica for male development time of the ECO-EVO2 treatment of experiment 2



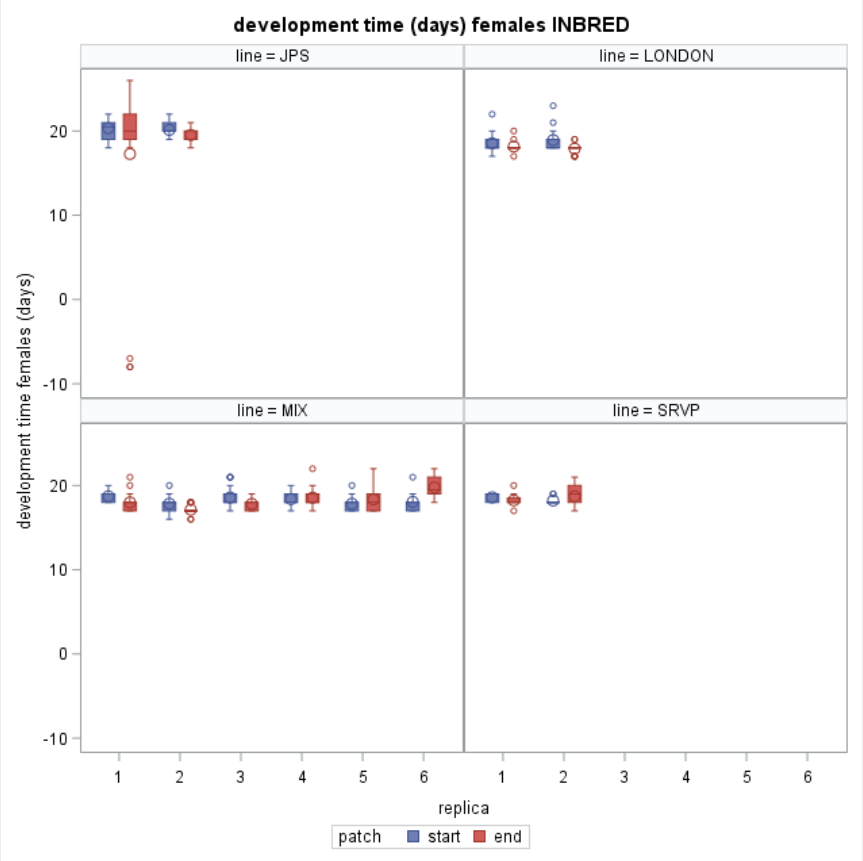
Appendix 1. 43: Effect sizes by replica for male development time of the ECO treatment of experiment 2



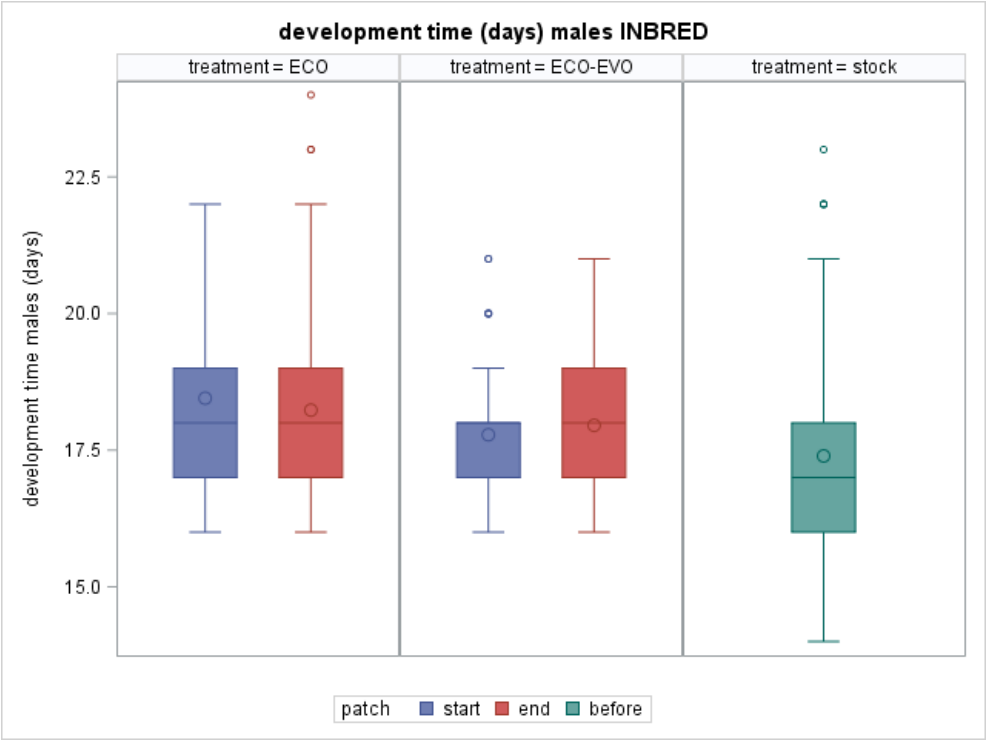
Appendix 1. 44: Female development time by treatment and patch for experiment 2



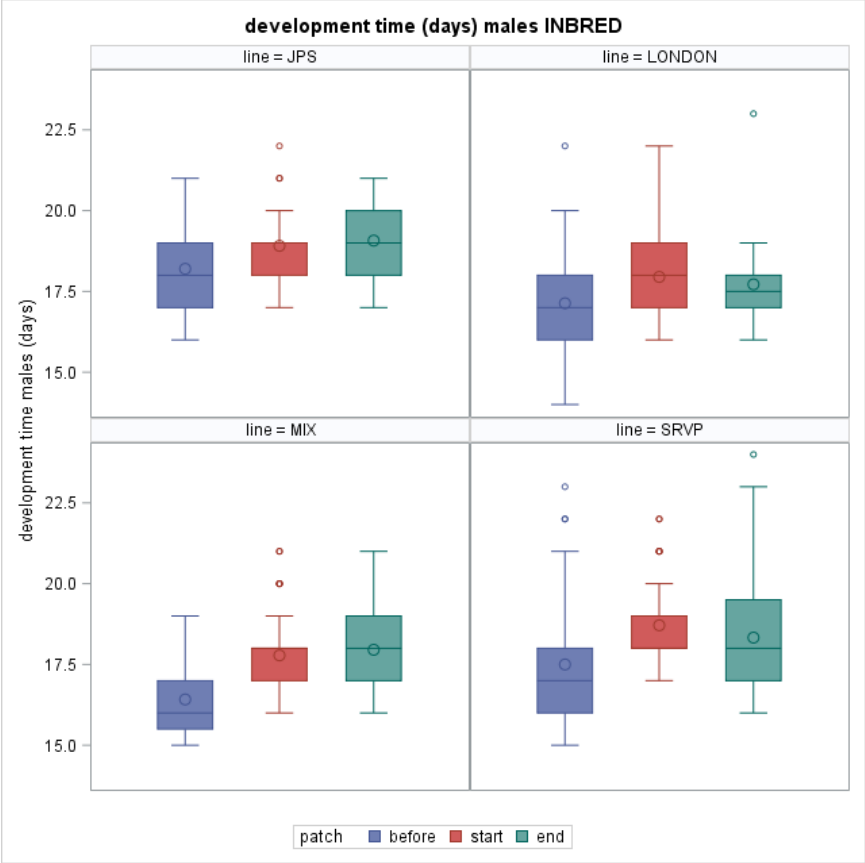
Appendix 1. 45: Female development time by line and patch for experiment 2



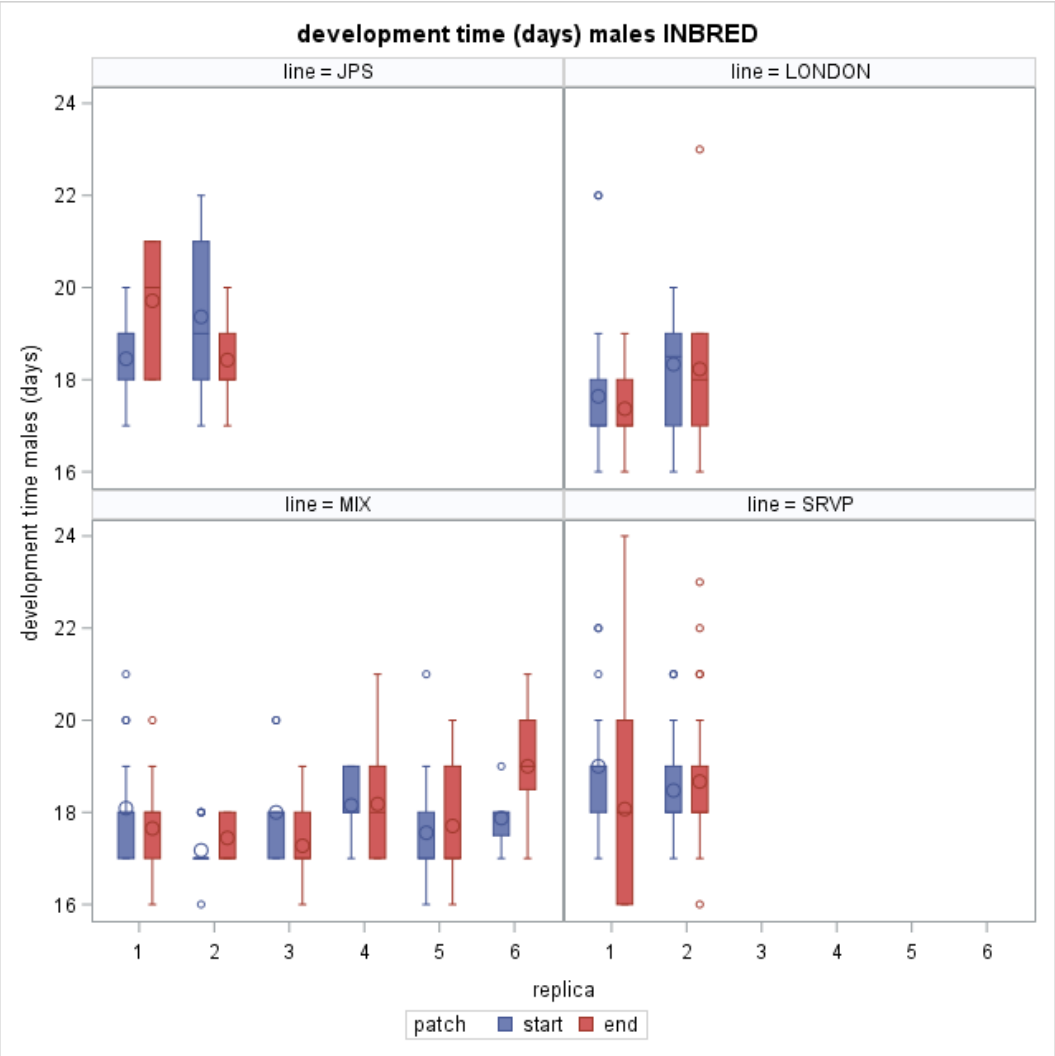
Appendix 1. 46: Female development time by replica and patch for experiment 2



Appendix 1. 47: Male development time by treatment and patch for experiment 2



Appendix 1. 48: Male development time by line and patch for experiment 2



Appendix 1. 49: Male development time by replica and patch for experiment 2

Sex ratio

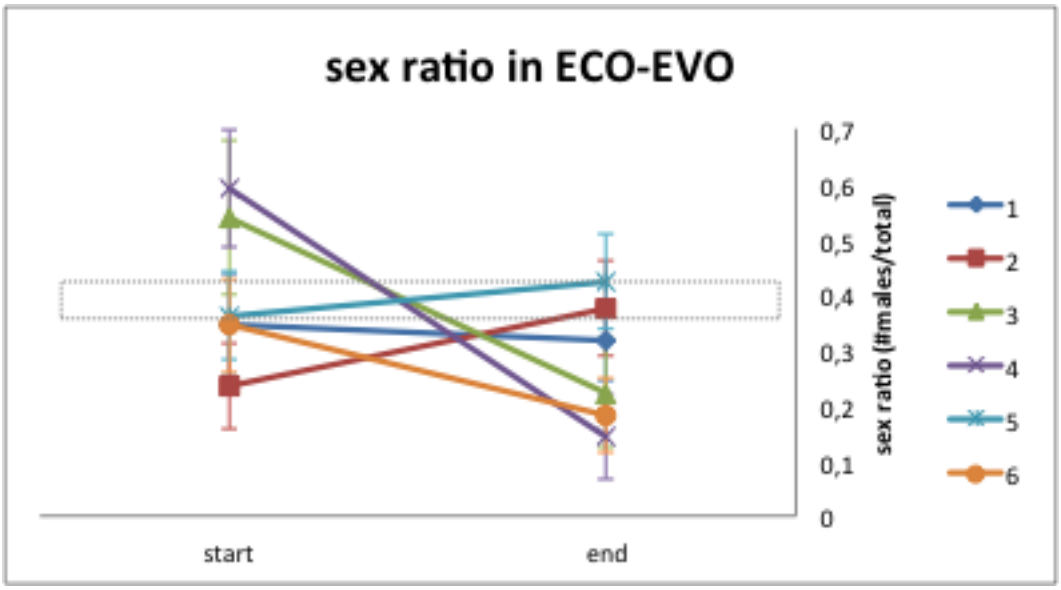
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,3.612}$=1.25; p=0.3332	$F_{1,3.362}$=0.46; p=0.5402
End-stock	$F_{1,5.497}$=0.70; p=0.4388	$F_{1,2.332}$=1.22; p=0.3710
Start-stock	$F_{1,2.345}$=0.01; p=0.9383	$F_{1,4.218}$=0.04; p=0.8514

Appendix 1. 50: Statistical output of sex ratio for experiment 1

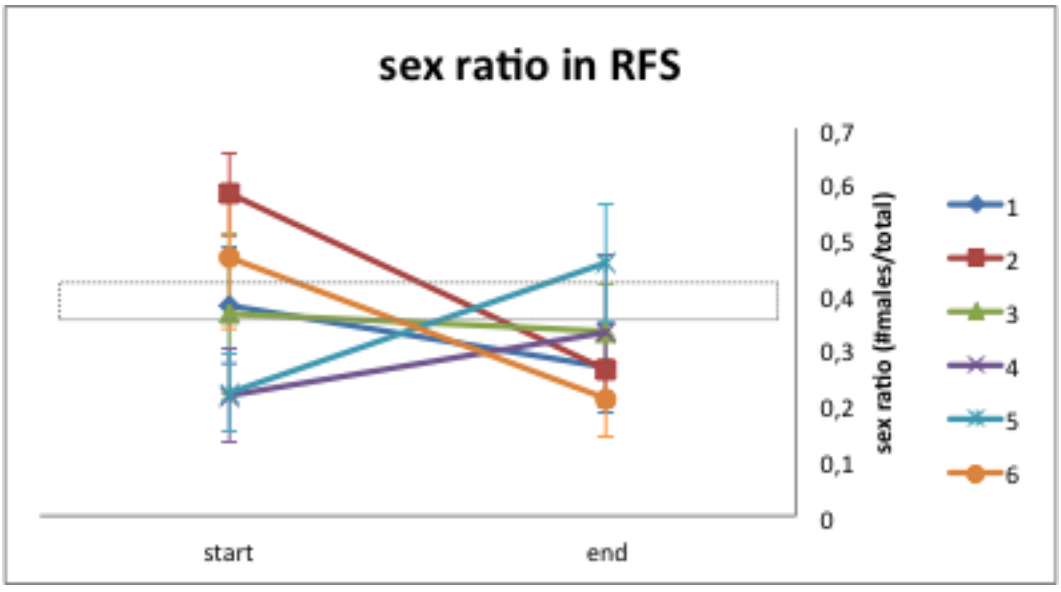
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,129.6}$=0.71; p=0.4018	$F_{1,5.734}$=0.98; p=0.3622
End-stock	$F_{1,71}$=0.22 p=0.6403	**$F_{1,7.322}$=6.34; p=0.0385**
Start-stock	$F_{1,6.288}$=0.58; p=0.4745	$F_{1,71}$=0.22 p=0.6403

Appendix 1. 51: Statistical output of sex ratio for experiment 2

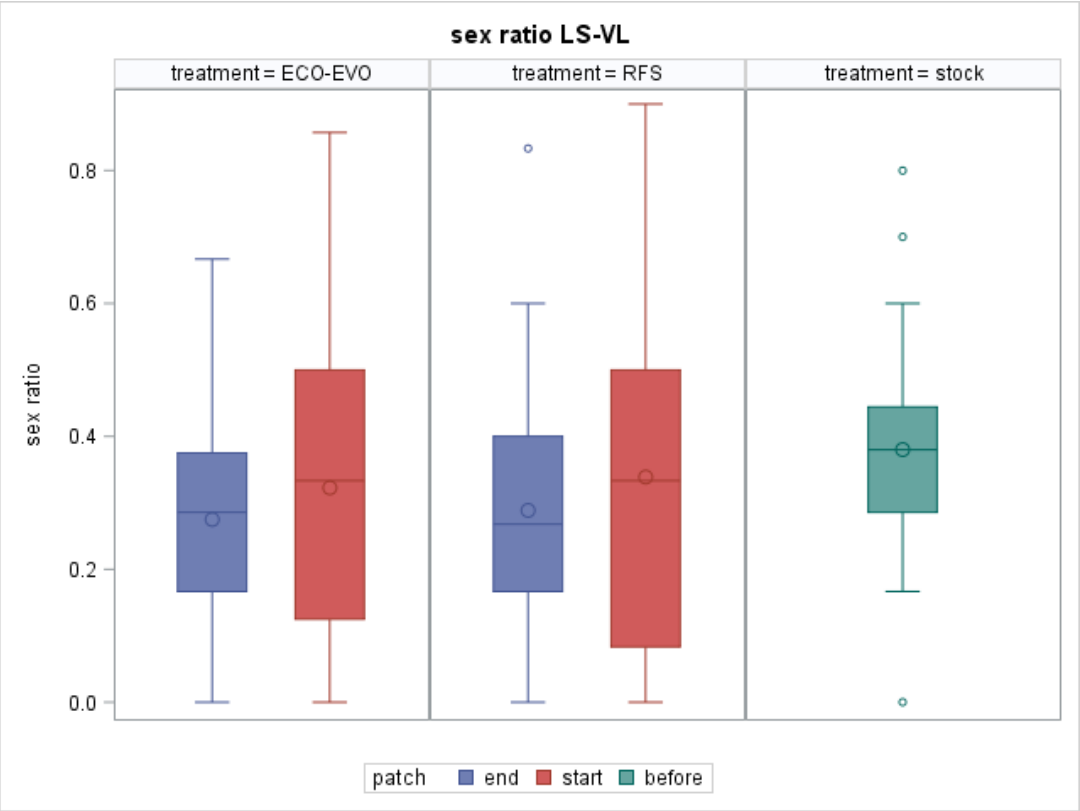
There are no consistent differences between stock populations and either the core or dispersal front populations. The only significant difference that could be observed was between the populations from the dispersal front and the stock population in the evolutionary constrained treatment of experiment 2 (ECO).



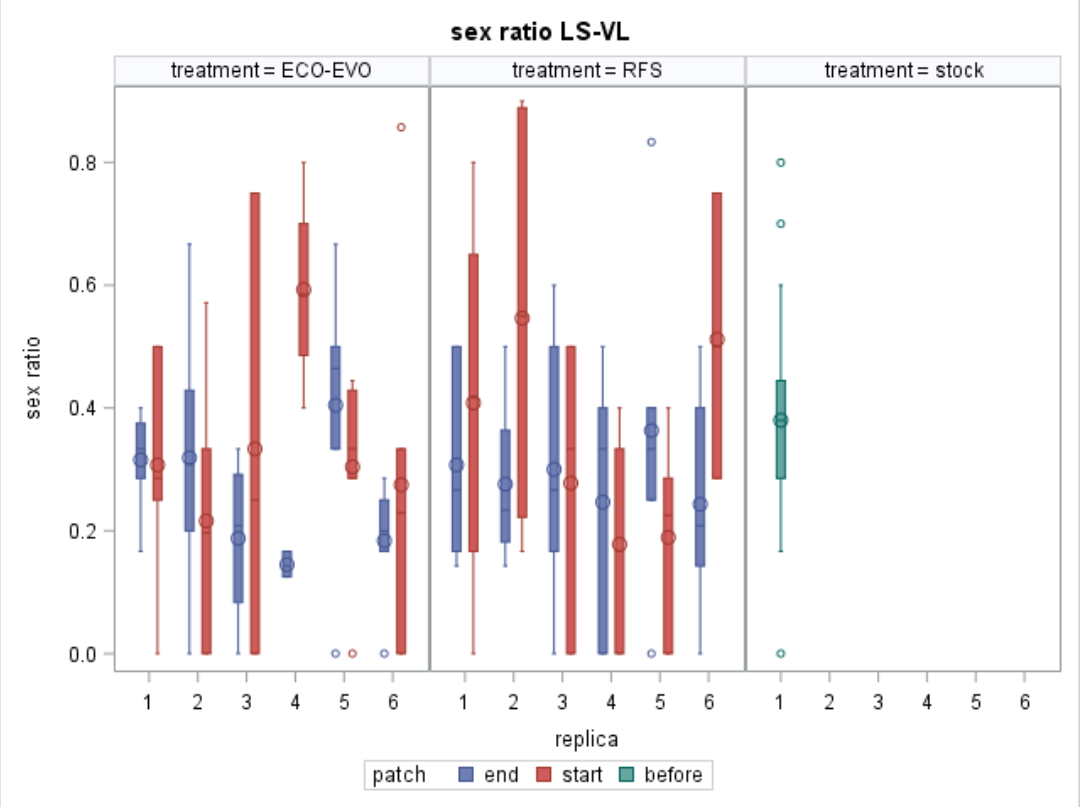
Appendix 1. 52: Effect sizes by replica for sex ratio of the ECO-EVO1 treatment of experiment 1



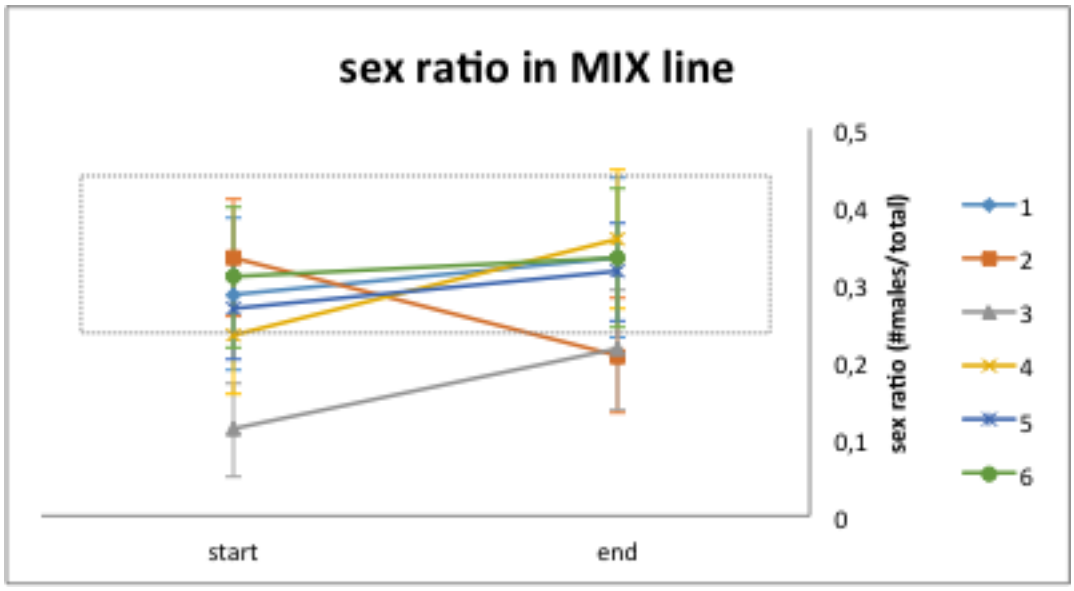
Appendix 1. 53: Effect sizes by replica for sex ratio of the RFS treatment of experiment 1



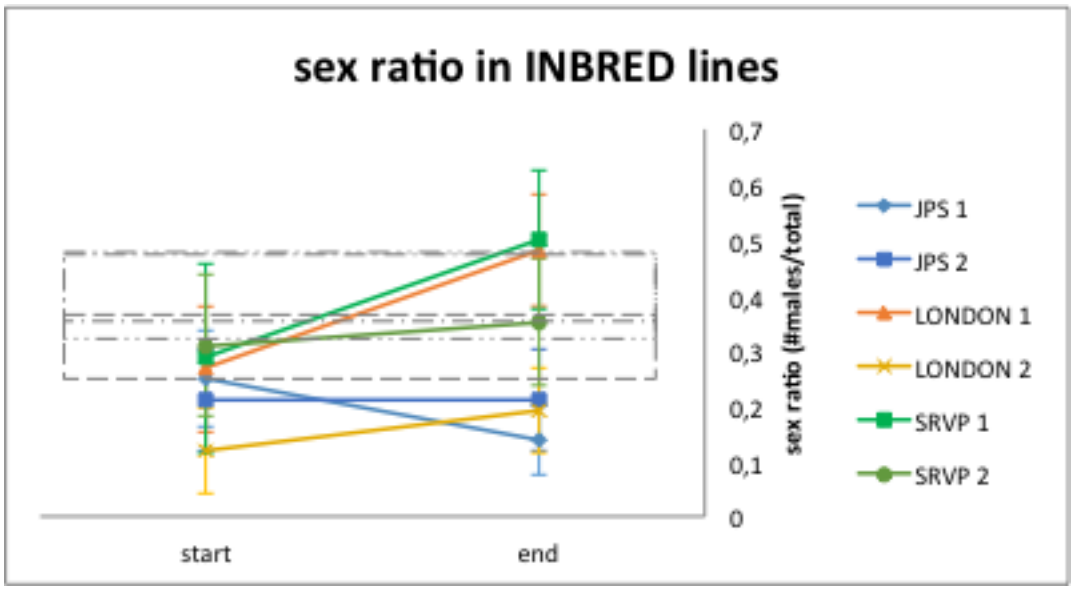
Appendix 1. 54: Sex ratio by treatment and patch for experiment 1



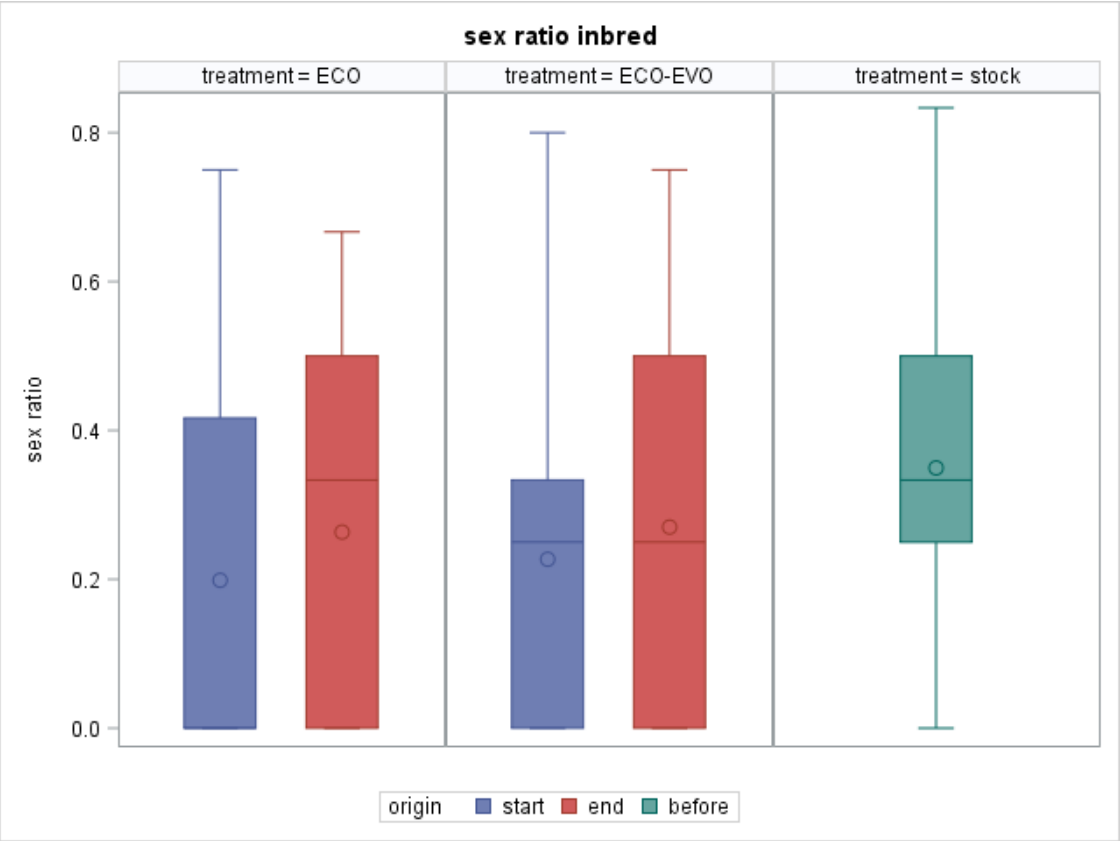
Appendix 1. 55: Sex ratio by replica and patch for experiment 1



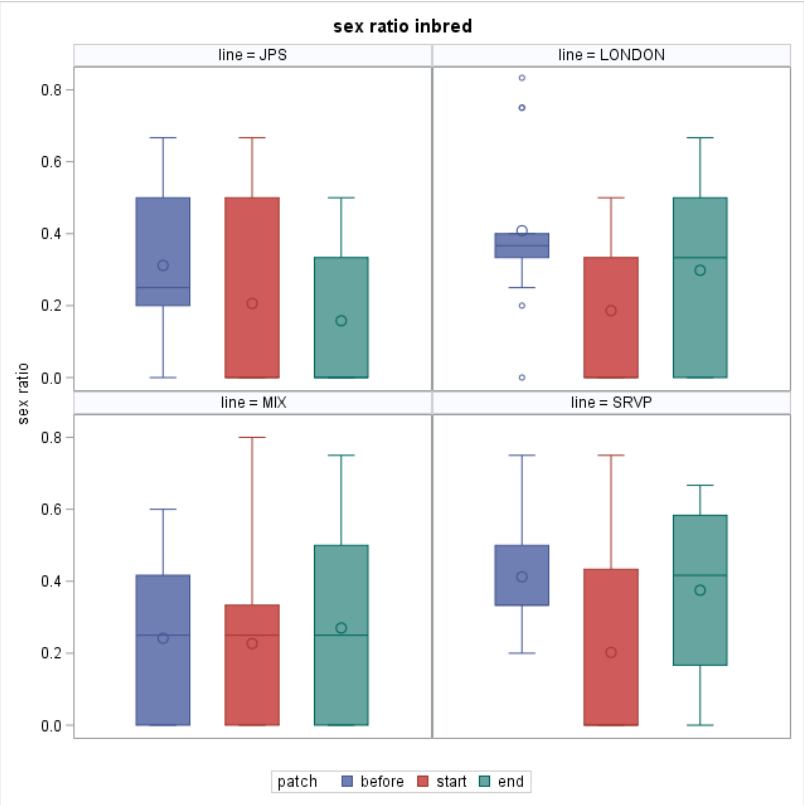
Appendix 1. 56: Effect sizes by replica for sex ratio of the ECO-EVO2 treatment of experiment 2



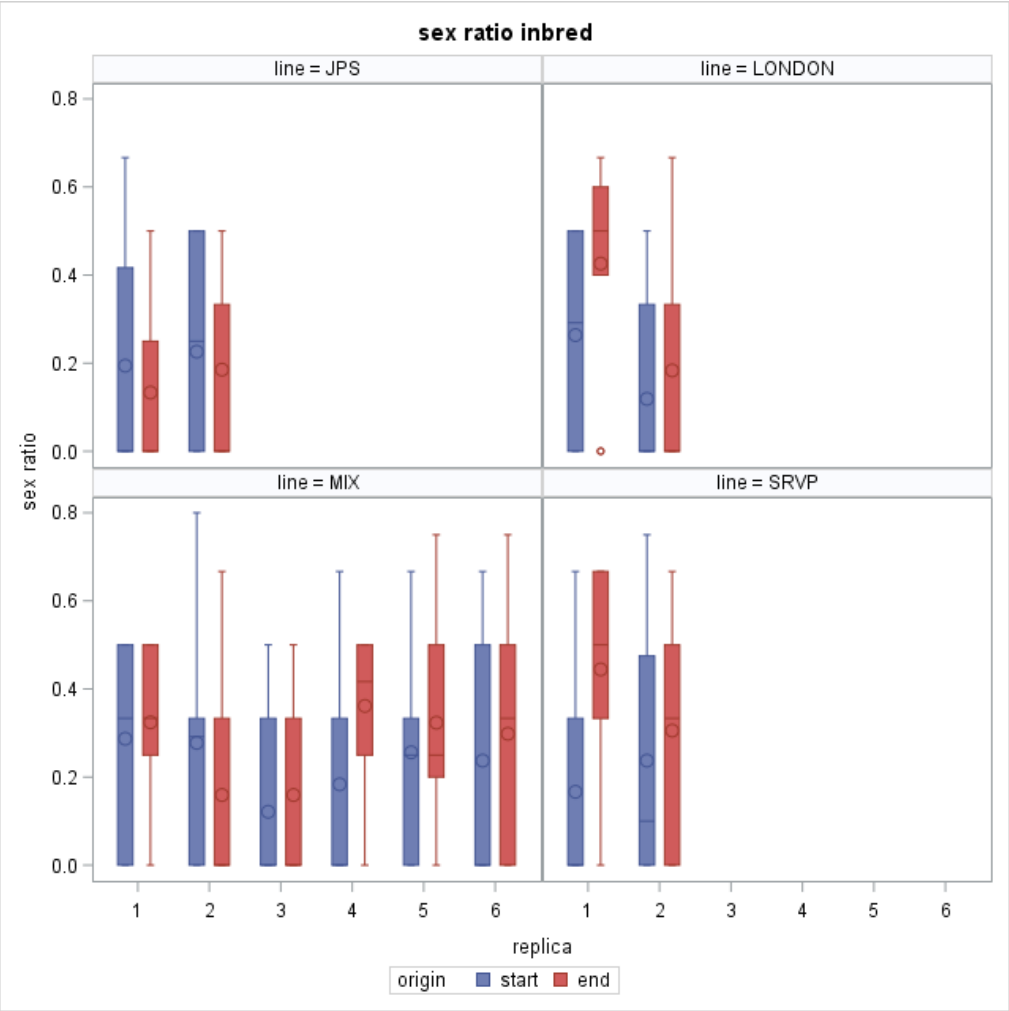
Appendix 1. 57: Effect sizes by replica for sex ratio of the ECO treatment of experiment 2



Appendix 1. 58: Sex ratio by treatment and patch for experiment 2



Appendix 1. 59: Sex ratio by line and patch for experiment 2



Appendix 1. 60: Sex ratio by replica and patch for experiment 2

Adult size

Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,3.48}$=0.52; p=0.5158	**$F_{1,131}$=6.07; p=0.0151**

Appendix 1. 61: Statistical output for adult size of experiment 1

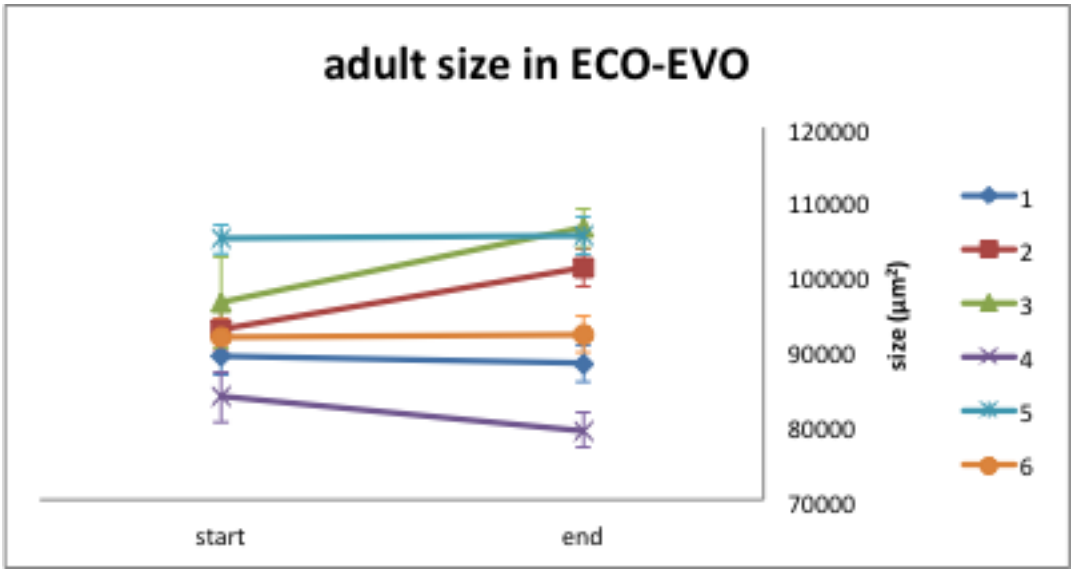
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,4.87}$=1.28; p=0.3104	$F_{1,4.66}$=0.52; p=0.5055
End-stock	$F_{1,6.1}$=0.04; p=0.8455	$F_{1,3.86}$=7.73; p=0.0518
Start-stock	$F_{1,8.97}$=0.04; p=0.8440	$F_{1,4.03}$=6.95; p=0.0573

Appendix 1. 62: Statistical output for adult size of experiment 2

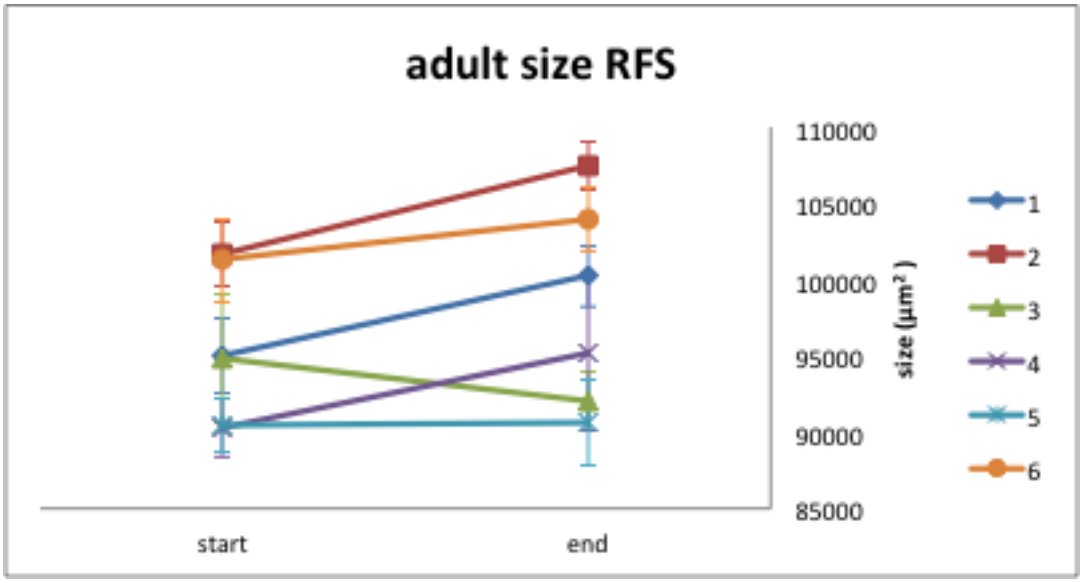
Due to an error in the timing of the pictures of the first experiment[14], comparison with stock populations was only possible for the second experiment. Whereas none of the comparisons

[14] Pictures of the stock individuals were taken three days after reaching the adult stage, whereas this was two days for the ECO-EVO and RFS treatments.

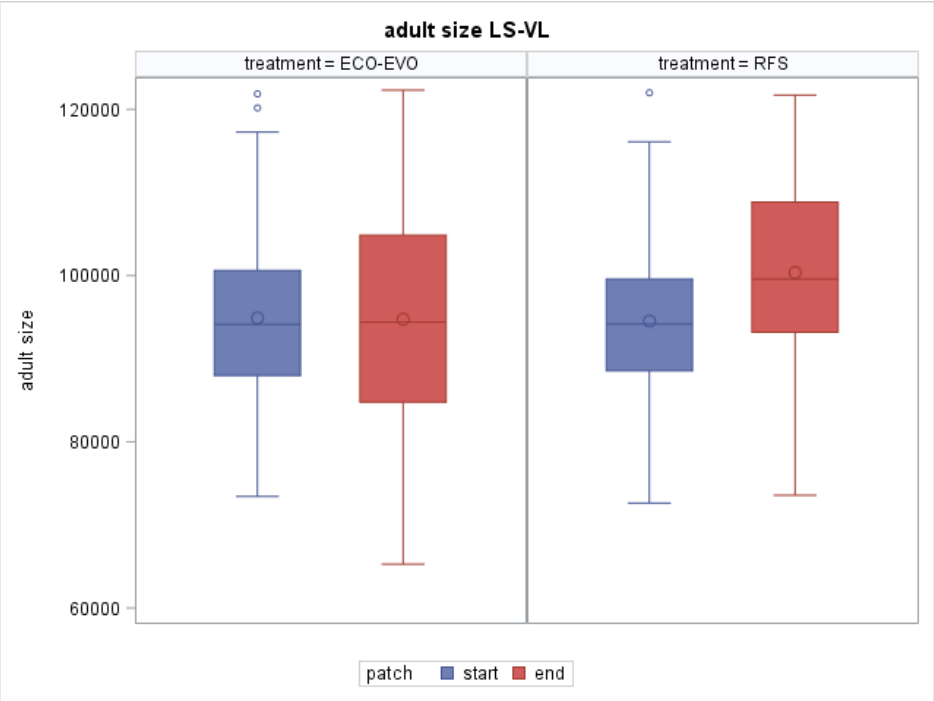
for the second experiment yielded significant results, it should be noted that stock population was marginally smaller compared to both core and dispersal front populations.



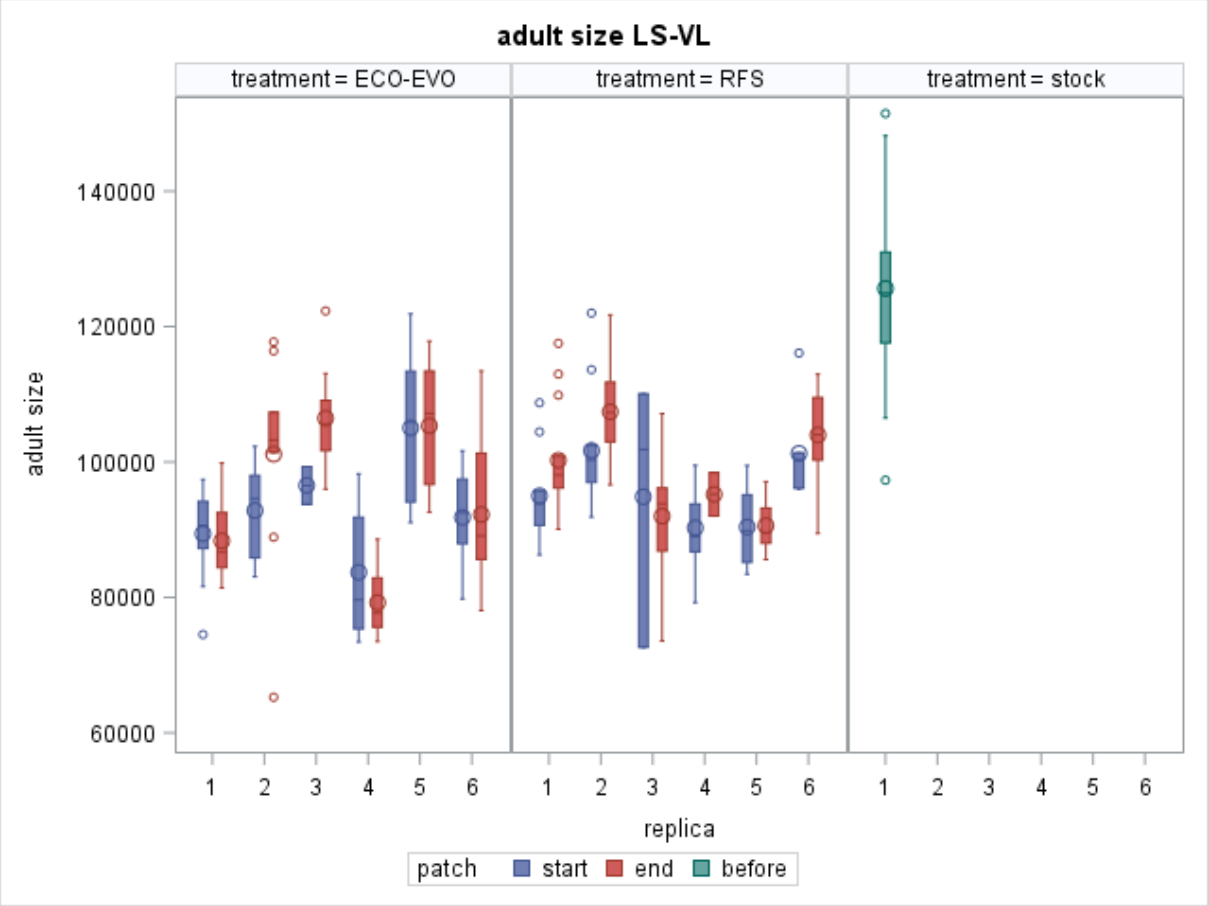
Appendix 1. 63: Effect sizes by replica for adult size of the ECO-EVO1 treatment of experiment 1



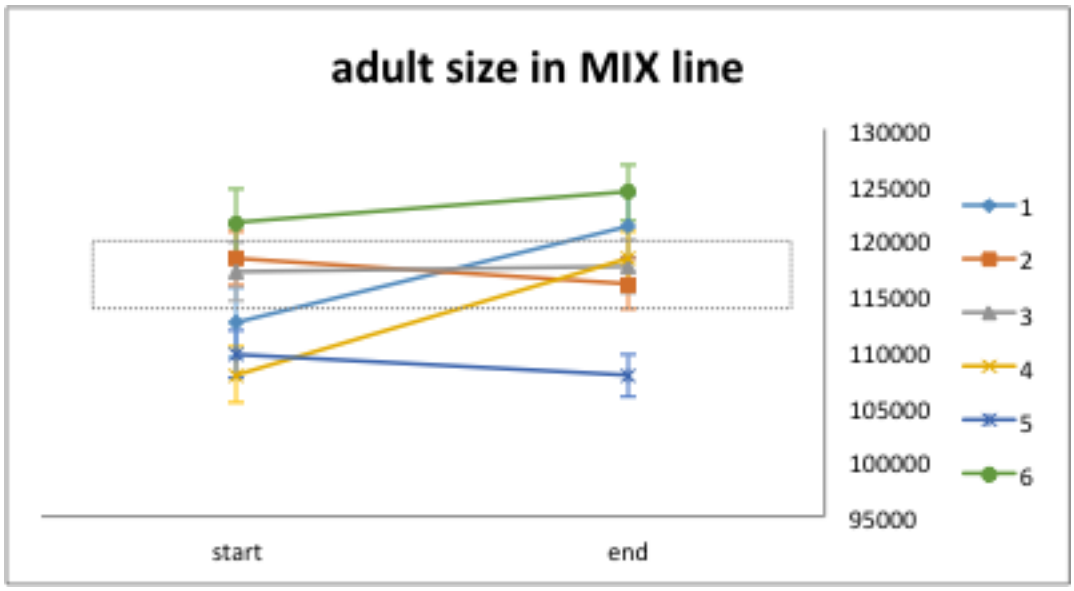
Appendix 1. 64: Effect sizes by replica for adult size of the RFS treatment of experiment 1



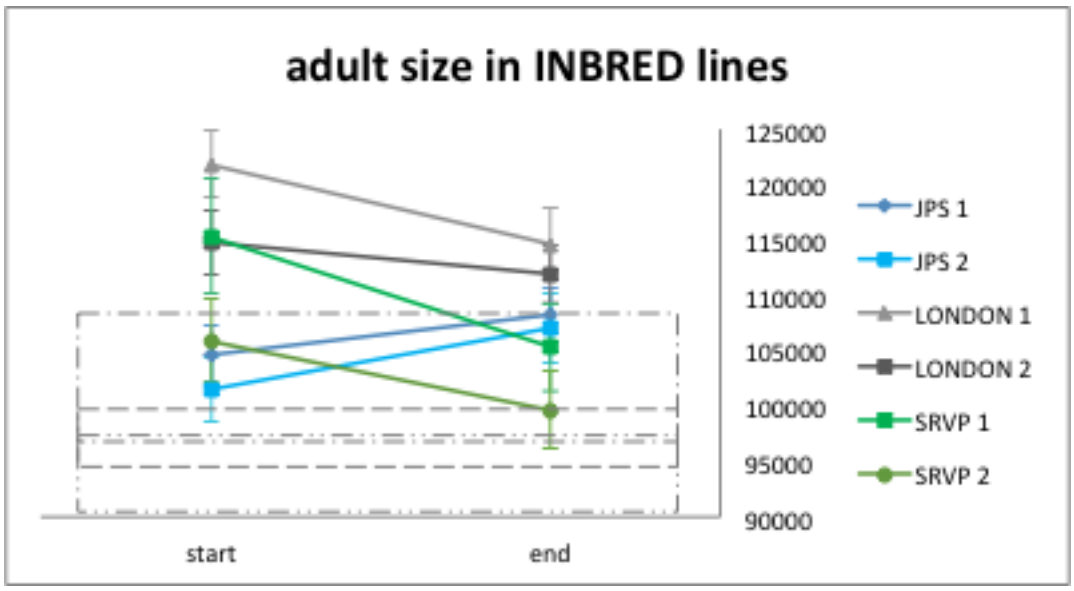
Appendix 1. 65: Adult size by treatment and patch for experiment 1



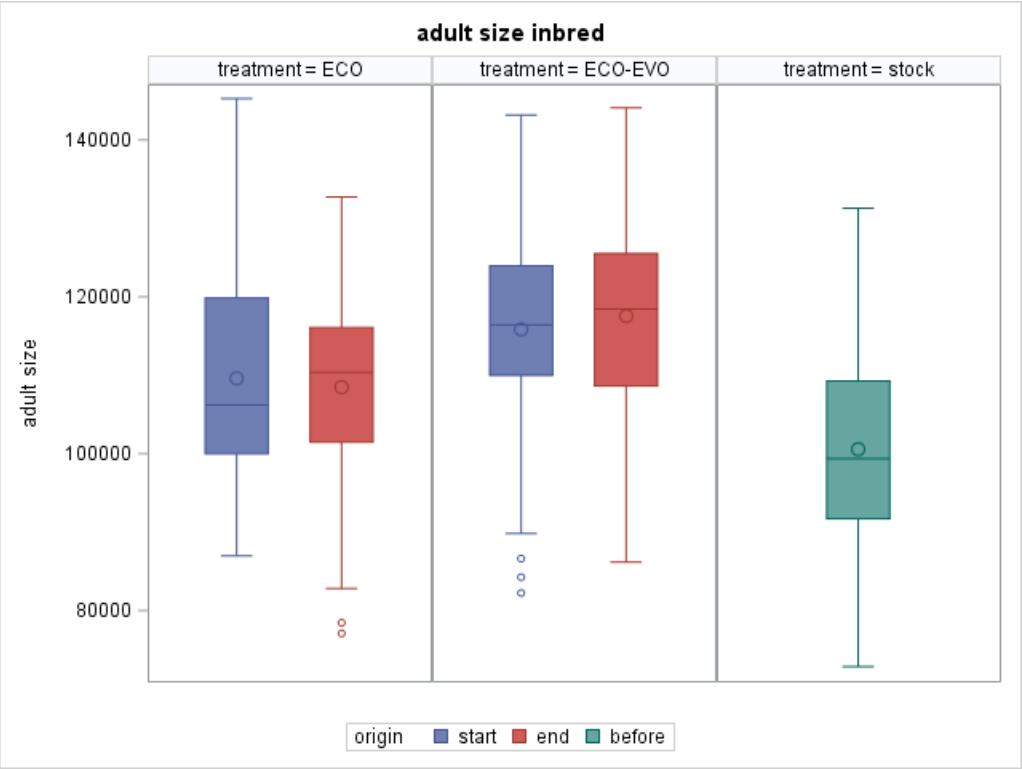
Appendix 1. 66: Adult size by replica and patch for experiment 1



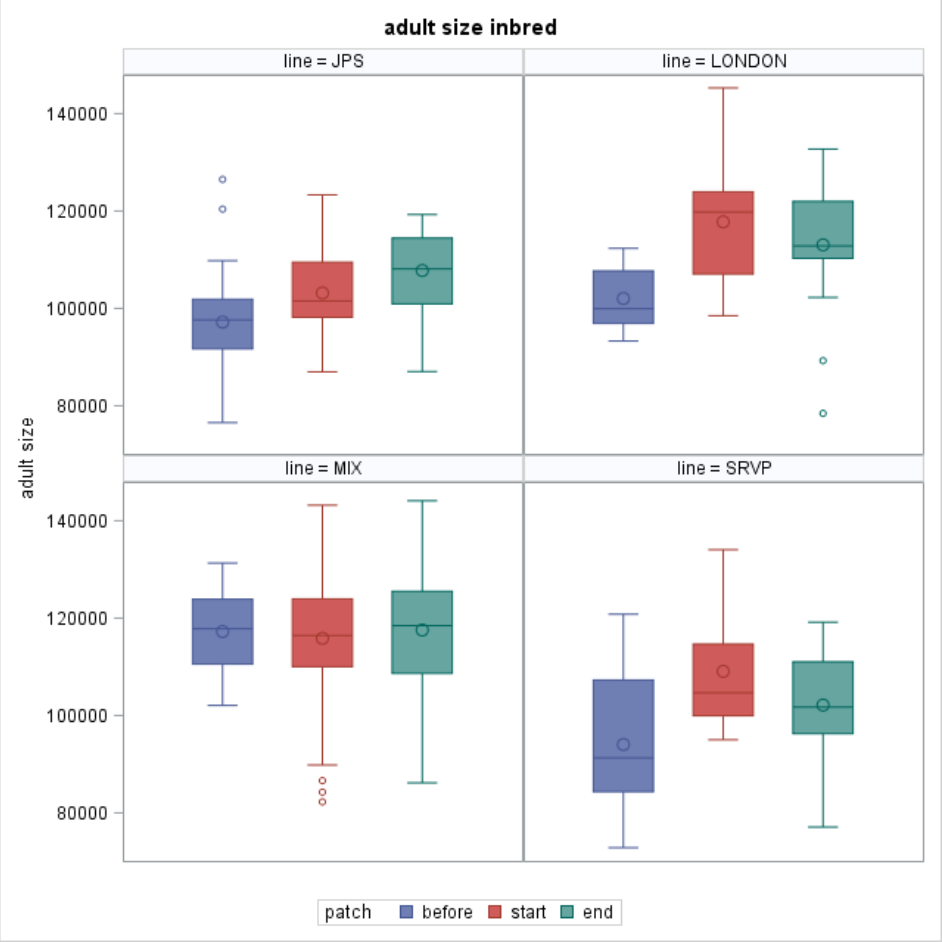
Appendix 1. 67: Effect sizes for adult size of the ECO-EVO2 treatment of experiment 2



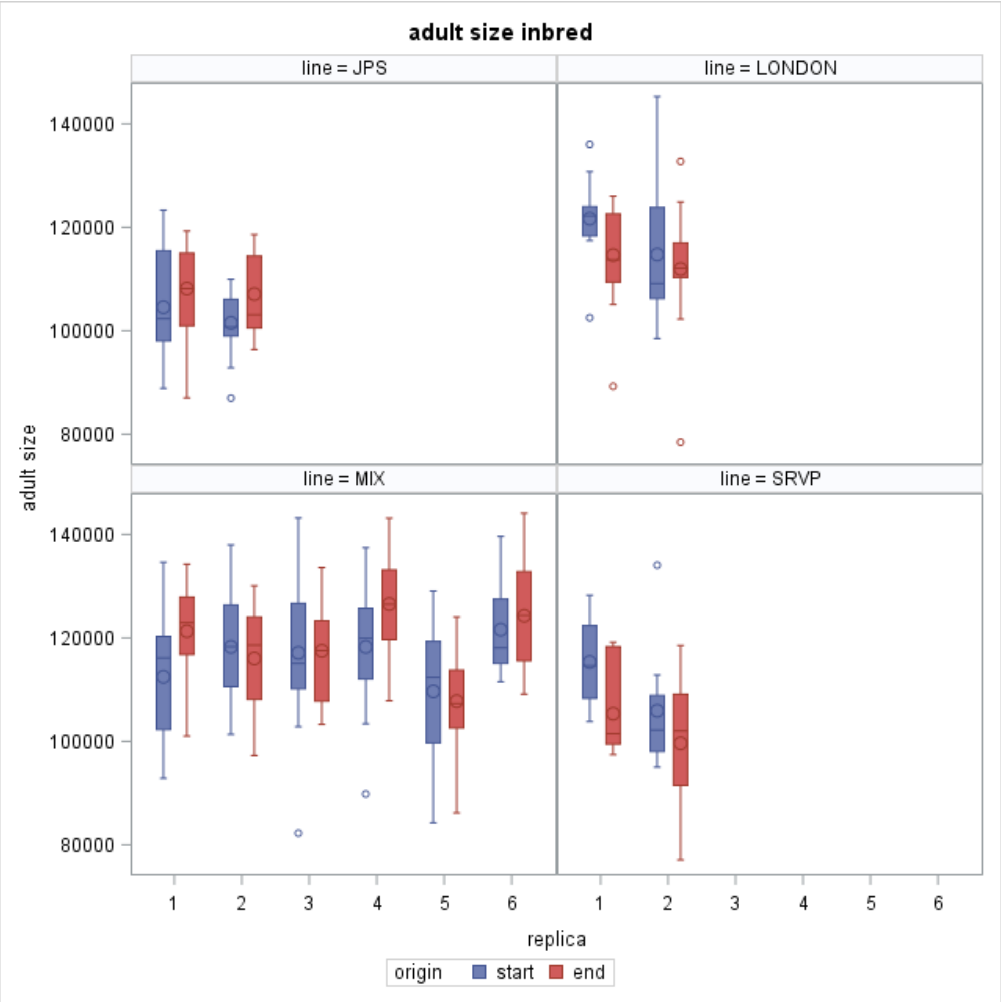
Appendix 1. 68: Effect sizes for adult size of the ECO treatment of experiment 2



Appendix 1. 69: Adult size by treatment and patch for experiment 2



Appendix 1. 70: Adult size by line and patch for experiment 2



Appendix 1. 71: Adult sizes by replica and patch for experiment 2

Lifetime fecundity

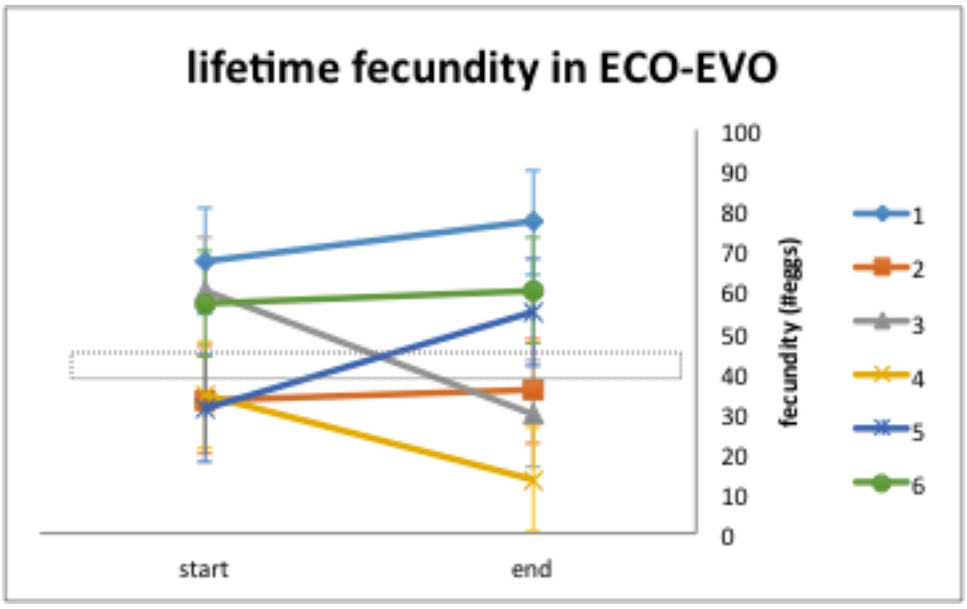
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,5}$=0.06; p=0.8118	$F_{1,4}$=1.69; p=0.2633
End-stock	$F_{1,3.52}$=0.03; p=0.8791	$F_{1,112}$=0.50; p=0.4822
Start-stock	$F_{1,2.37}$=0.16; p=0.7222	$F_{1,7.08}$=0.53; p=0.4889

Appendix 1. 72: Statistical output for lifetime fecundity of experiment 1

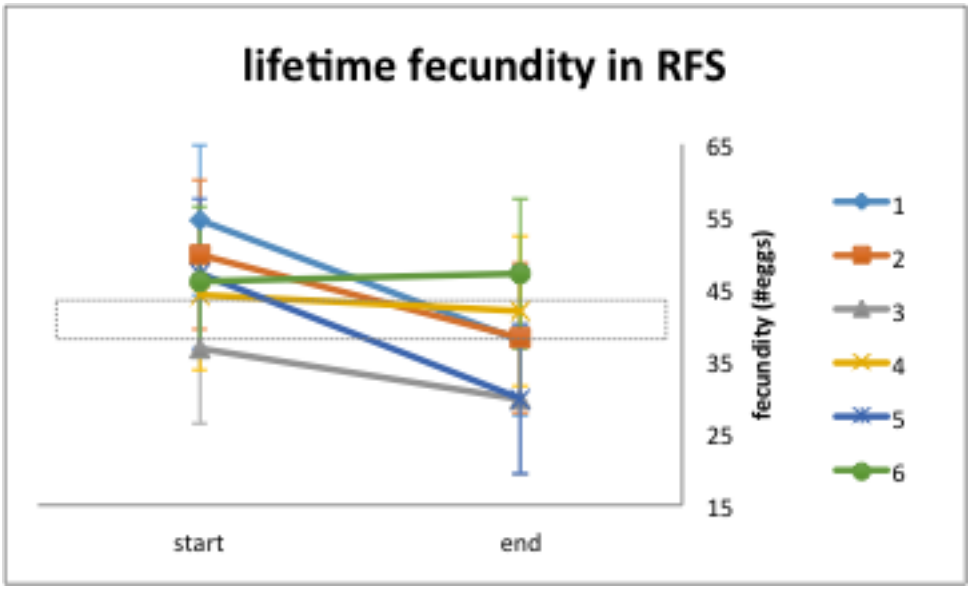
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,3.85}$=0.13; p=0.7398	$F_{1,101}$=0.04; p=0.8456
End-stock	$F_{1,4.83}$=1.42 p=0.2839	$F_{1,4.26}$=0.71; p=0.4433
Start-stock	$F_{1,4.6}$=0.74; p=0.4322	$F_{1,6.53}$=0.54; p=0.4888

Appendix 1. 73: Statistical output of lifetime fecundity for experiment 2

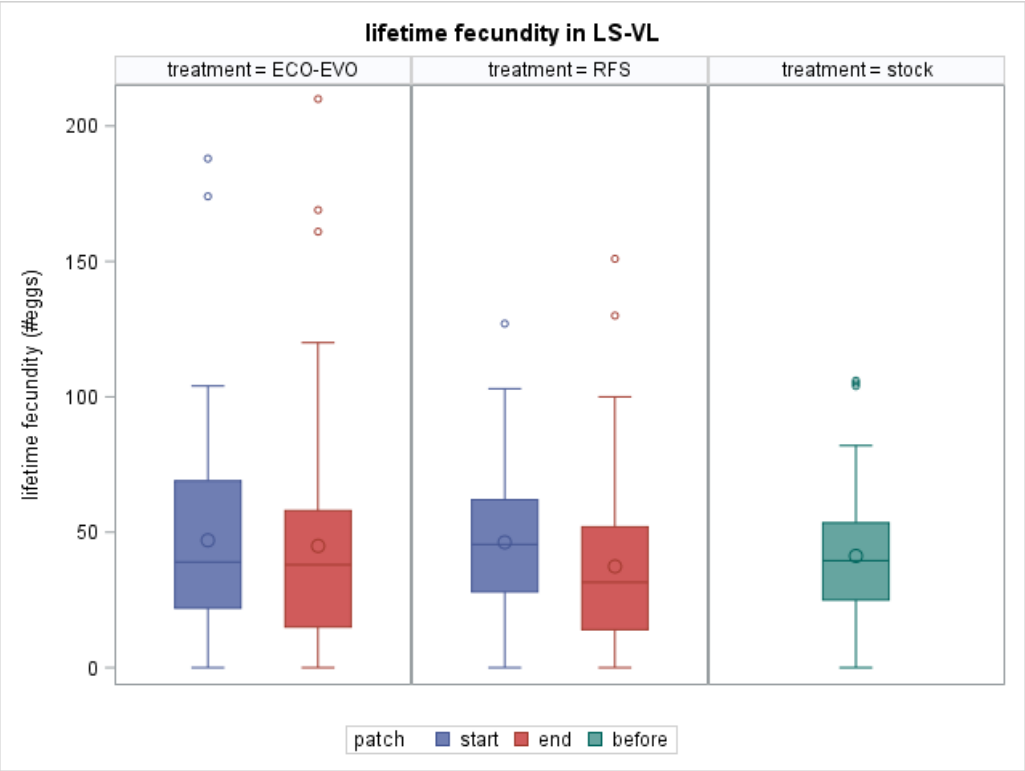
There were no significant differences between core or dispersal front populations with the stock in any of the treatments of both experiments.



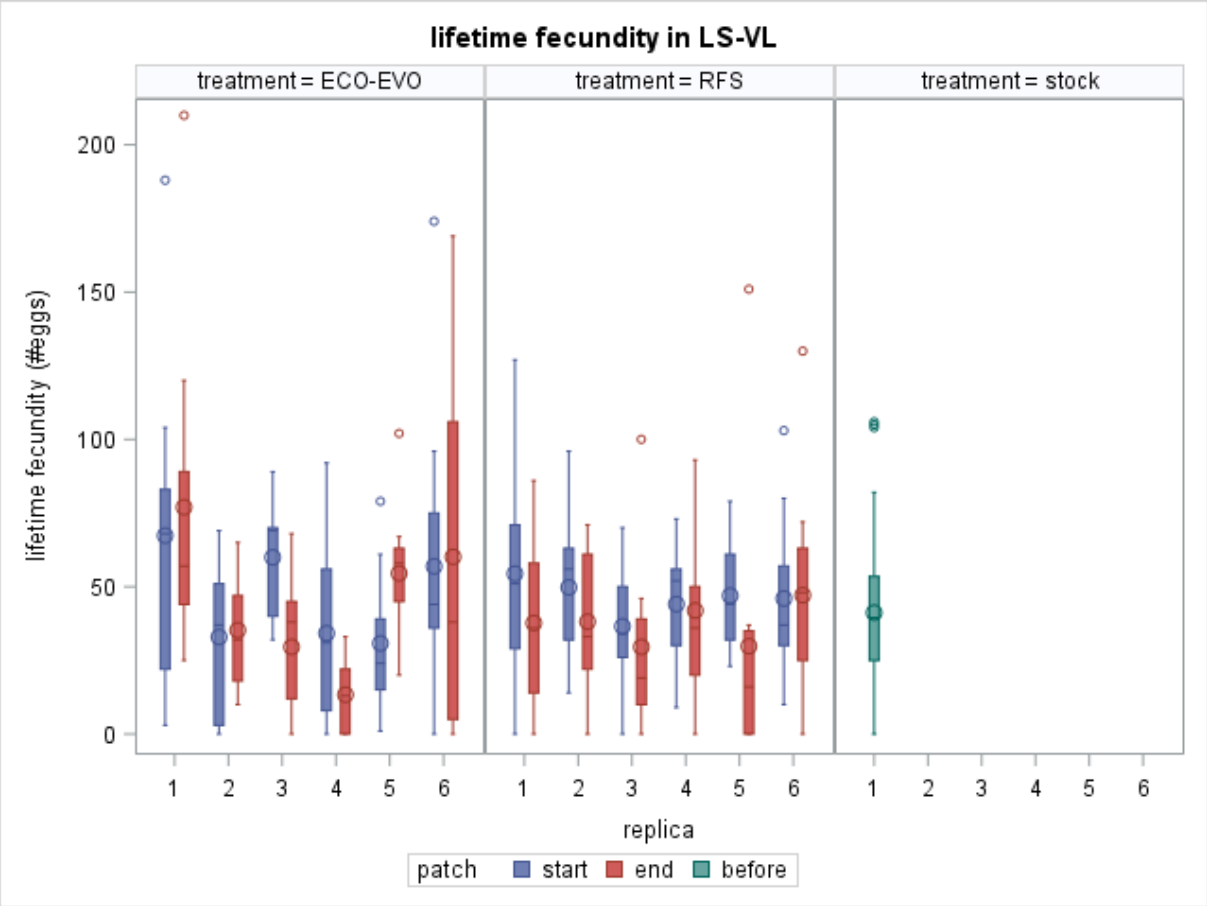
Appendix 1. 74: Effect sizes by replica for lifetime fecundity of the ECO-EVO1 treatment of experiment 1



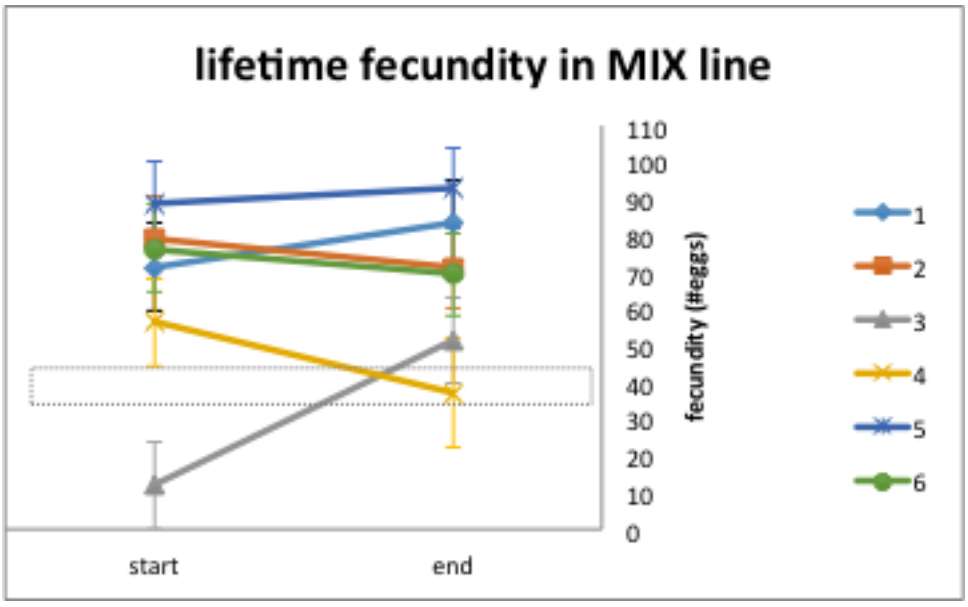
Appendix 1. 75: Effect sizes by replica for lifetime fecundity of the RFS treatment of experiment 1



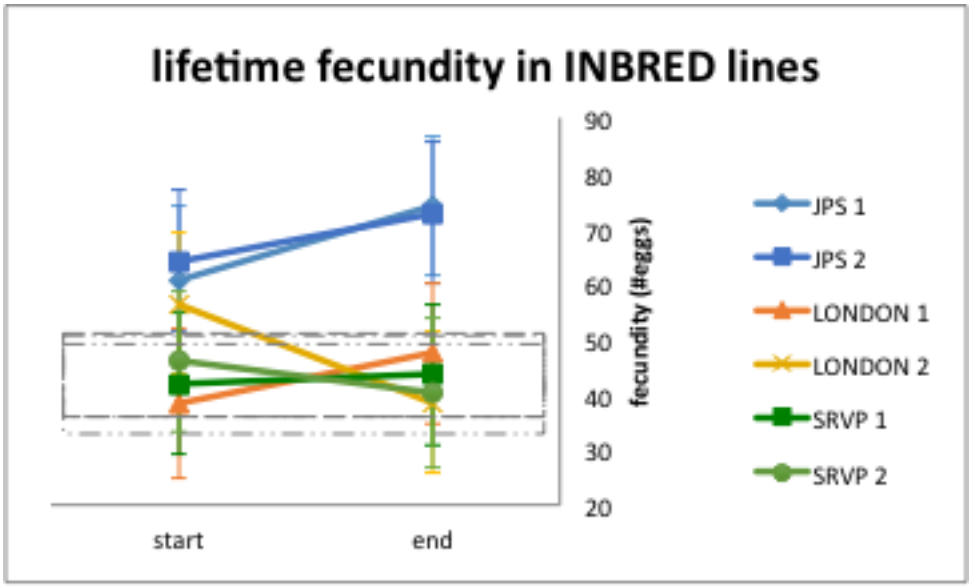
Appendix 1. 76: Lifetime fecundity by treatment and patch for experiment 1



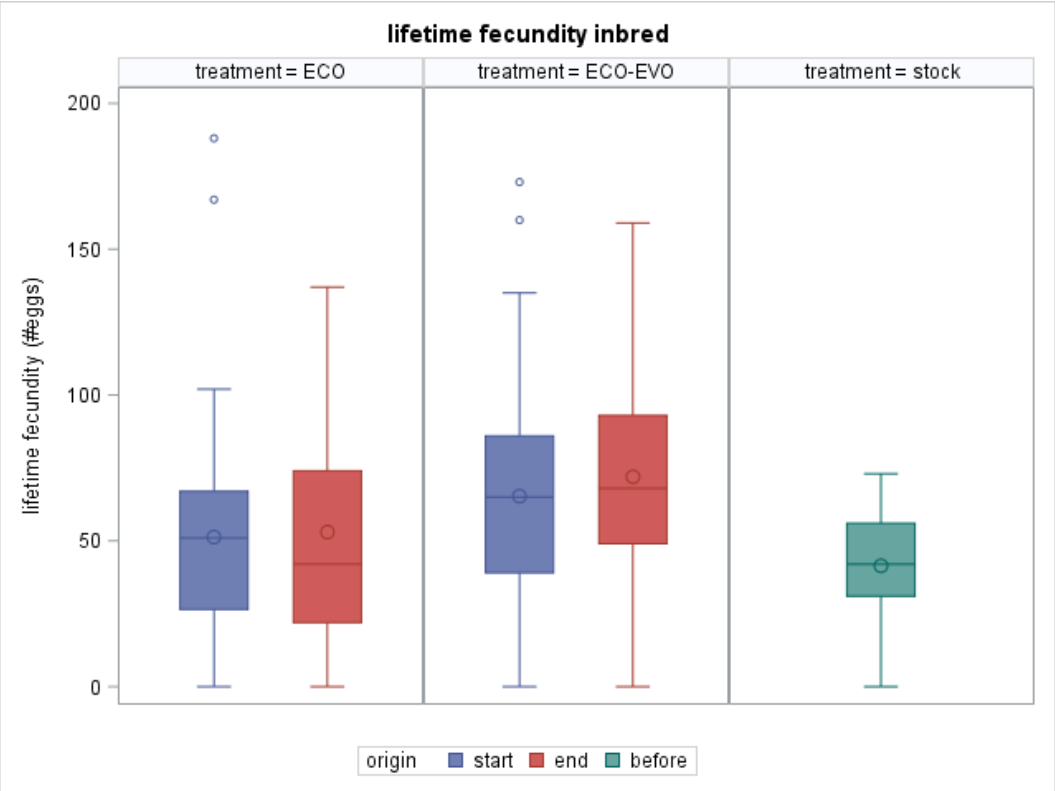
Appendix 1. 77: Lifetime fecundity by replica and patch for experiment 1



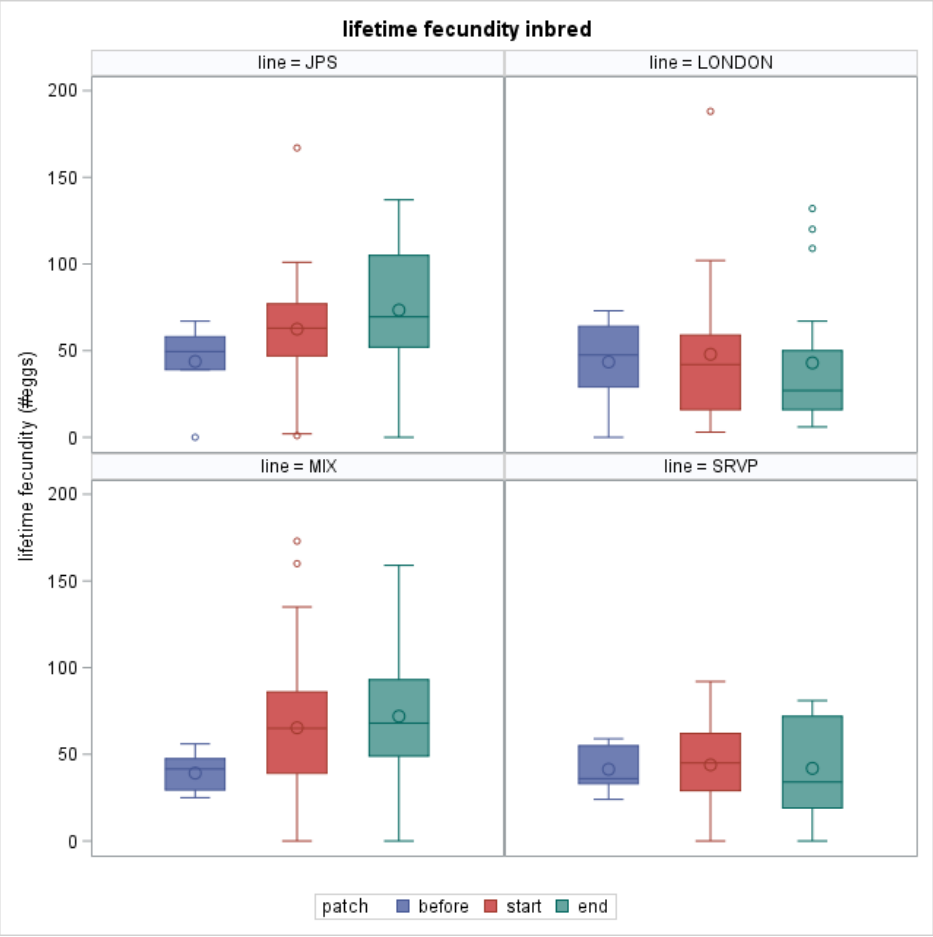
Appendix 1. 78: Effect sizes by replica for lifetime fecundity of the ECO-EVO2 treatment of experiment 2



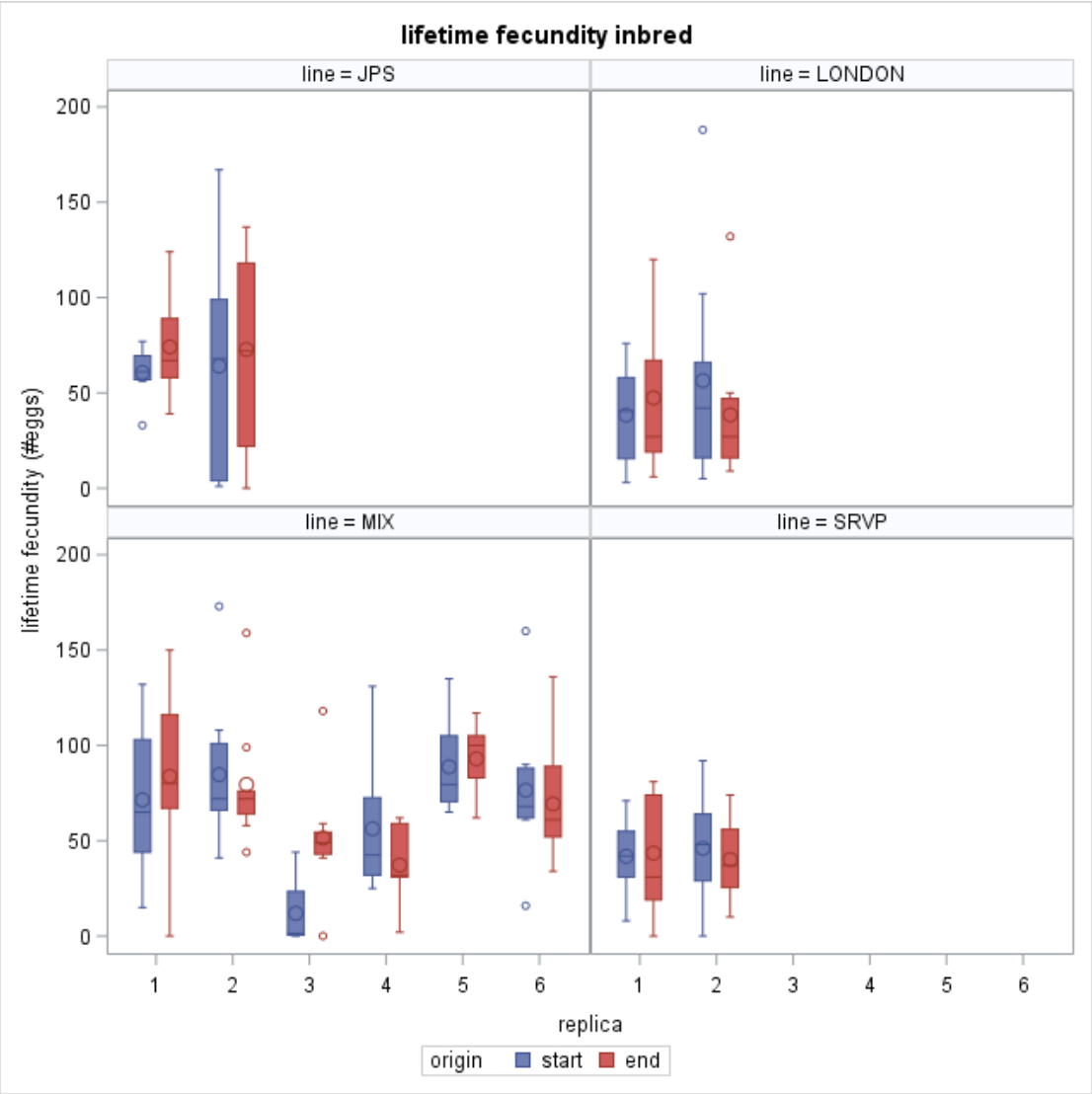
Appendix 1. 79: Effect sizes by replica for lifetime fecundity of the ECO treatment of experiment 2



Appendix 1. 80: Lifetime fecundity by treatment and patch for experiment 2



Appendix 1. 81: Lifetime fecundity by line and patch for experiment 2



Appendix 1. 82: Lifetime fecundity by replica and patch for experiment 2

Mean daily fecundity

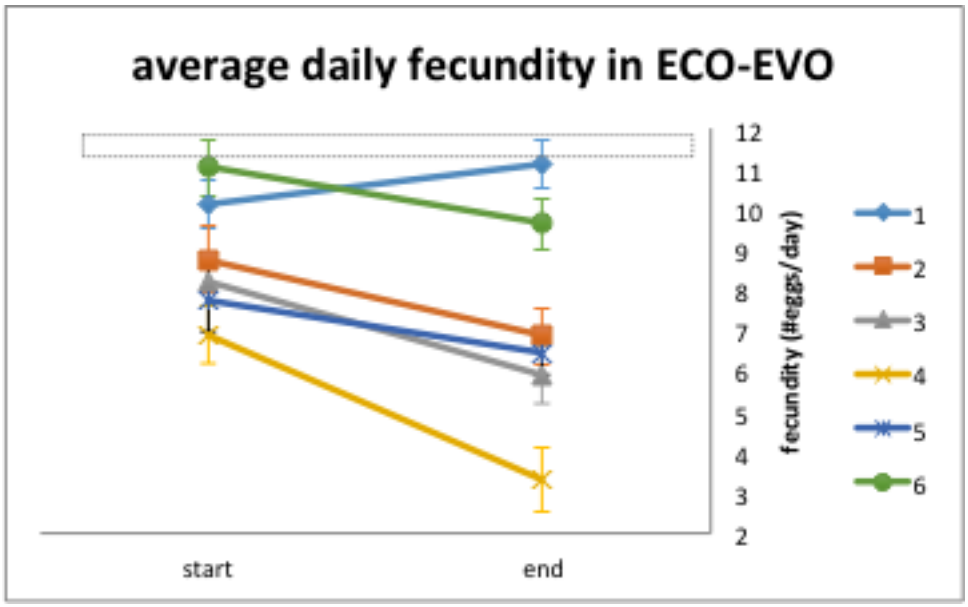
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,5.21}$=4.42; p=0.0872	$F_{1,5.04}$=1.56; p=0.2666
End-stock	$F_{1,5.12}$=1.98; p=0.2176	**$F_{1,5.87}$=15.23; p=0.0083**
Start-stock	$F_{1,5.54}$=2.18; p=0.1939	**$F_{1,8.02}$=8.86; p=0.0176**

Appendix 1. 83: Statistical output of mean daily fecundity for experiment 1

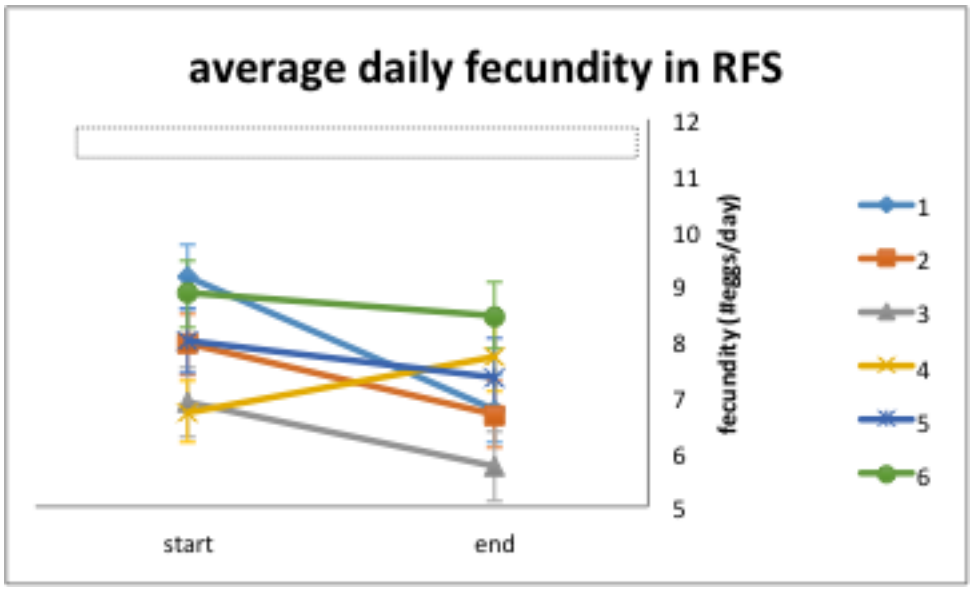
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,5.62}$=0.04; p=0.8458	$F_{1,6.15}$=3.42; p=0.1129
End-stock	$F_{1,4.82}$=0.55 p=0.4915	$F_{1,2.39}$=4.86; p=0.1372
Start-stock	$F_{1,5.03}$=0.51; p=0.5066	$F_{1,6.99}$=1.16; p=0.3168

Appendix 1. 84: Statistical output of mean daily fecundity for experiment 2

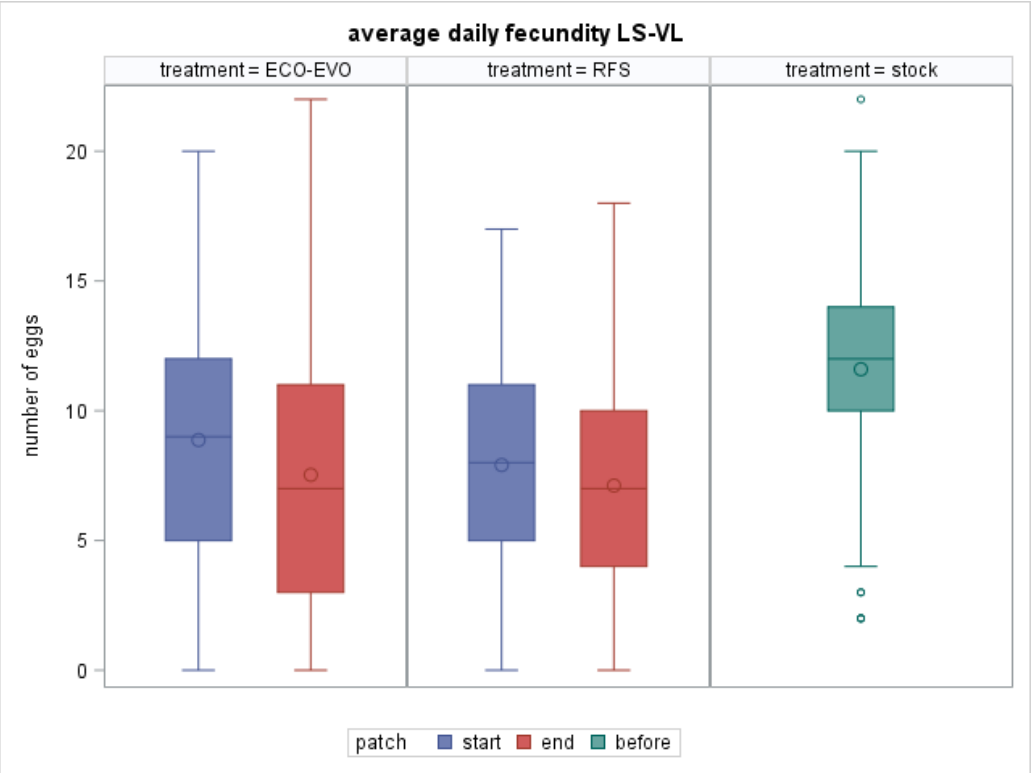
Whereas there were no significant differences between core and dispersal front populations in any of the treatments, there was a trend towards a lower average daily fecundity for both core and dispersal front in the evolutionary constrained (RFS) treatment of experiment 1, compared to the stock population. This trend did however only occur in the first experiment, but not in the second.



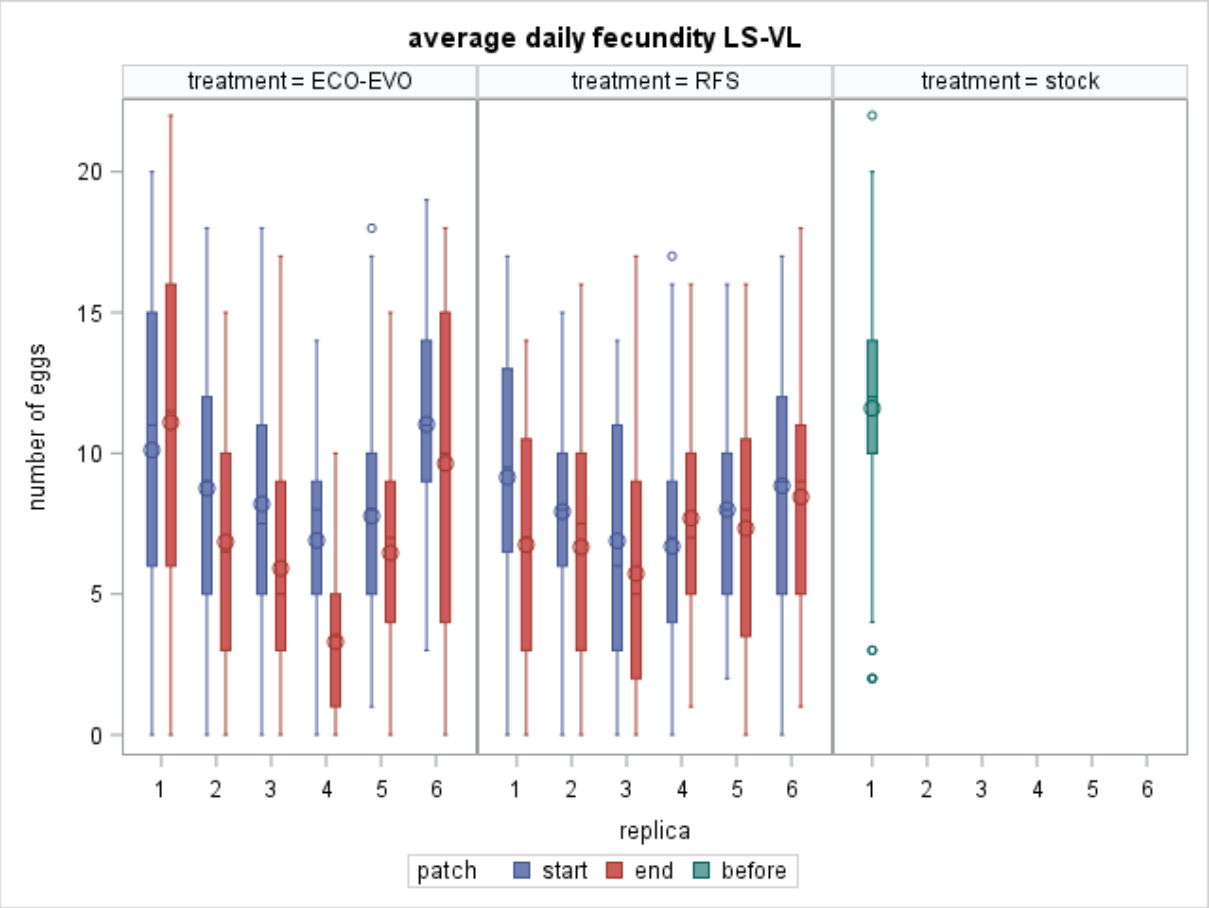
Appendix 1. 85: Effect sizes by replica for mean daily fecundity of the ECO-EVO1 treatment of experiment 1



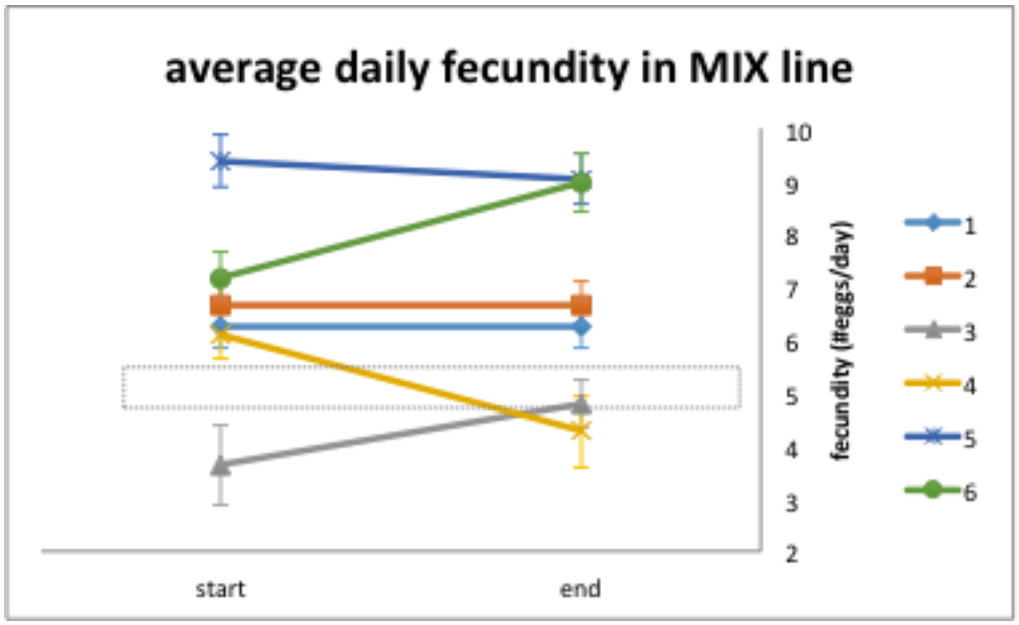
Appendix 1. 86: Effect sizes by replica for mean daily fecundity of the RFS treatment of experiment 1



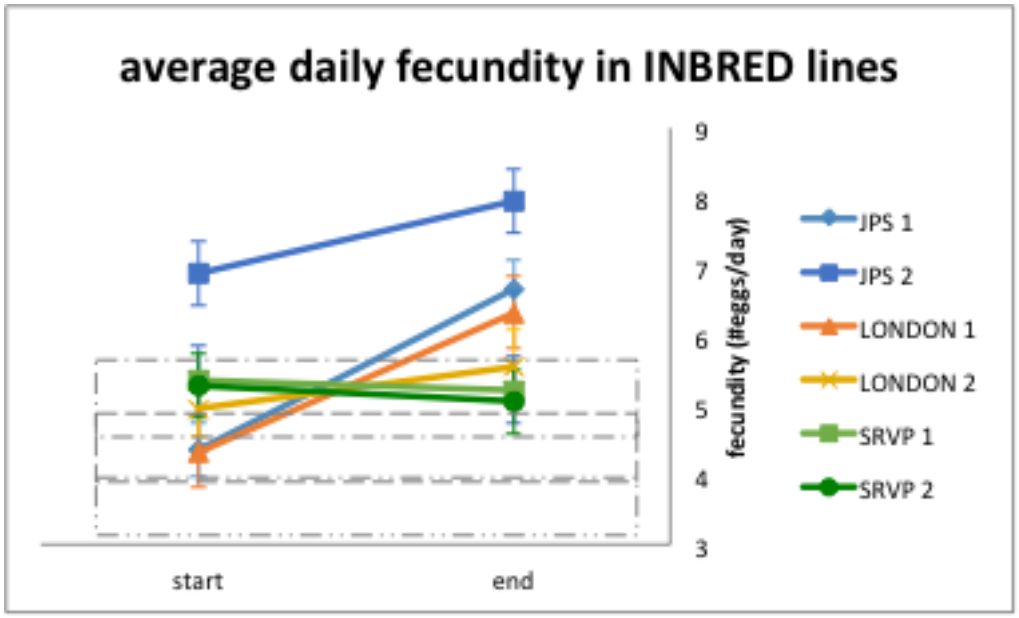
Appendix 1. 87: Mean daily fecundity by treatment and patch for experiment 1



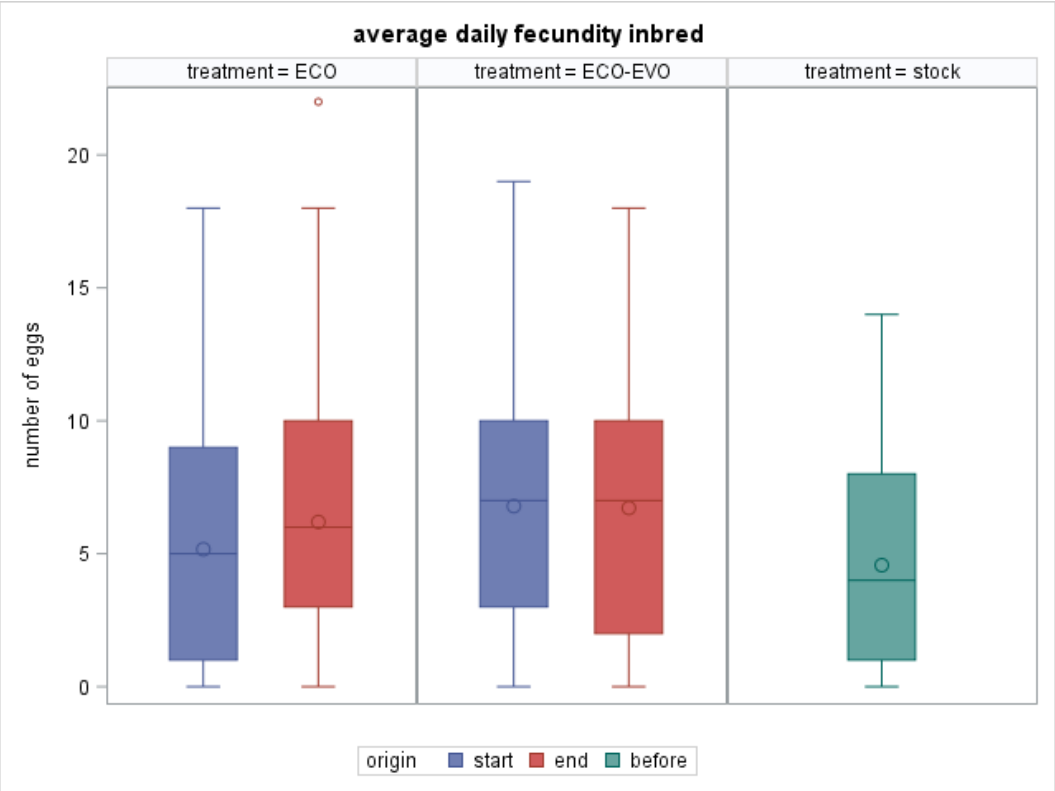
Appendix 1. 88: Mean daily fecundity by replica and patch for experiment 1



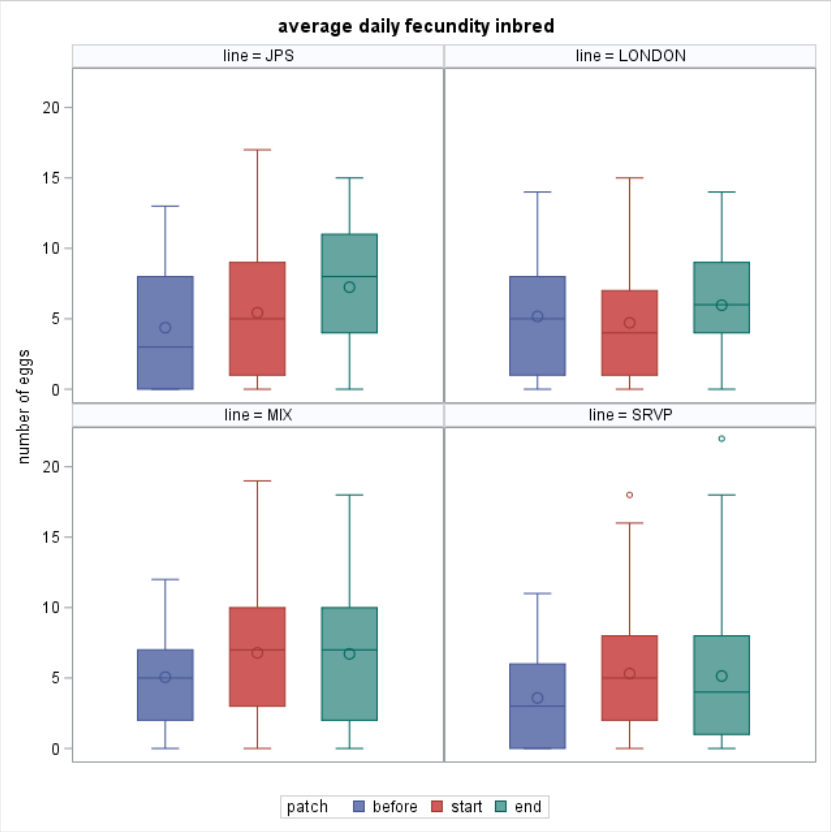
Appendix 1. 89: Effect sizes by replica for mean daily fecundity of the ECO-EVO2 treatment of experiment 2



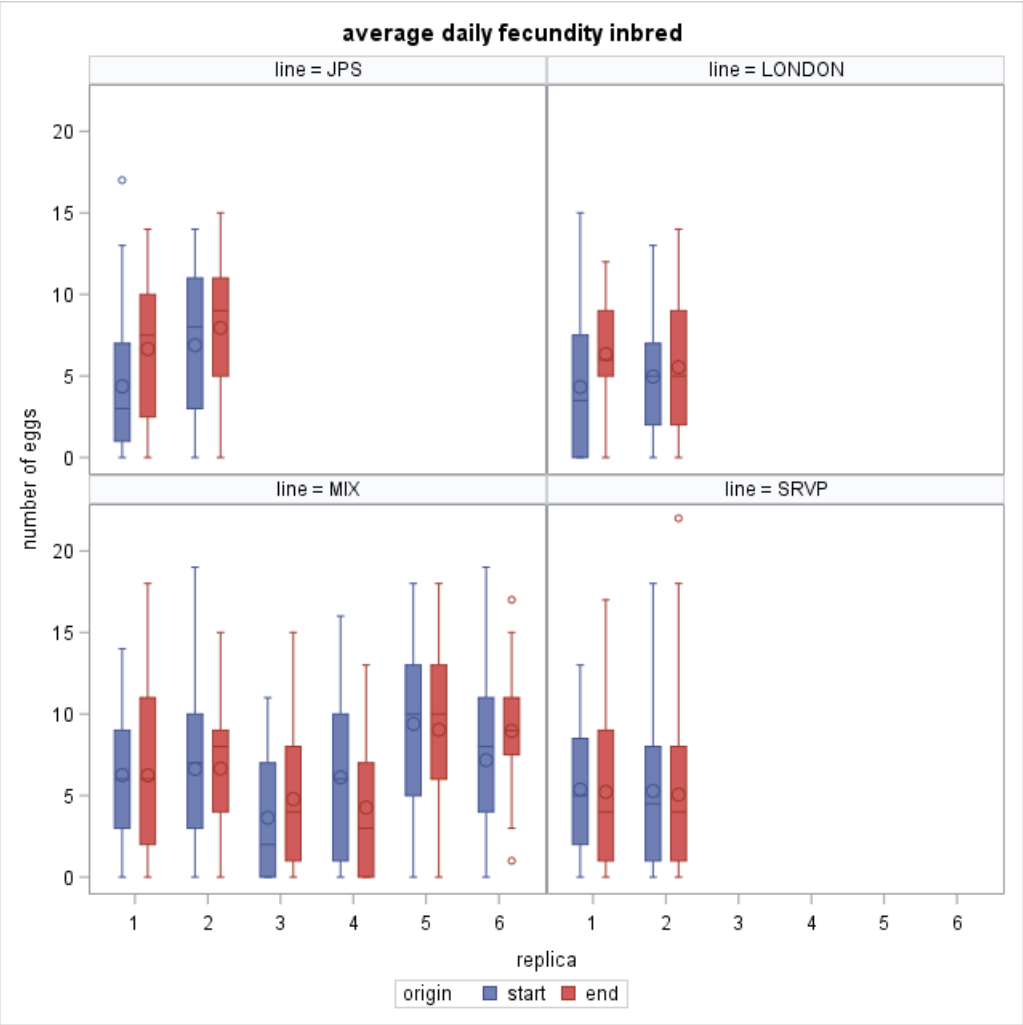
Appendix 1. 90: Effect sizes by replica for mean daily fecundity of the ECO treatment of experiment 2



Appendix 1. 91: Mean daily fecundity by treatment and patch for experiment 2



Appendix 1. 92: Mean daily fecundity by line and patch for experiment 2



Appendix 1. 93: Mean daily fecundity by replica and patch for experiment 2

Cumulative fecundity

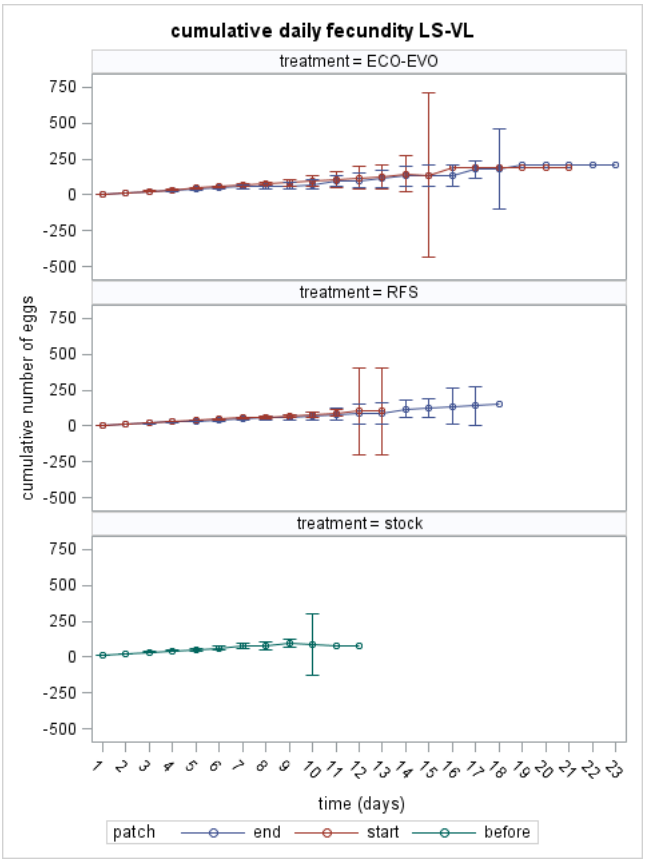
Experiment 1	ECO-EVO1	RFS
Start-end	Slope: $F_{1,668}$=0.20; p=0.6575 Intercept: $F_{1,9.95}$=0.20; p=0.6624	**Slope: $F_{1,641}$=5.33; p=0.0212** Intercept: $F_{1,11.6}$=1.69; p=0.2192
End-stock	Slope: **$F_{1,623}$=21.77; p<0.0001** Intercept: $F_{1,5.27}$=0.72; p=0.4315	Slope: $F_{1,569}$=3.75; p=0.0533 Intercept: $F_{1,7.28}$=2.40; p=0.1633
Start-stock	Slope: **$F_{1,571}$=45.76; p<0.0001** Intercept: $F_{1,6.31}$=1.48; p=0.2666	Slope: $F_{1,592}$=0.00; p=0.9472 Intercept: $F_{1,11.6}$=1.15; p=0.3052

Appendix 1. 94: statistical output of cumulative fecundity for experiment 1

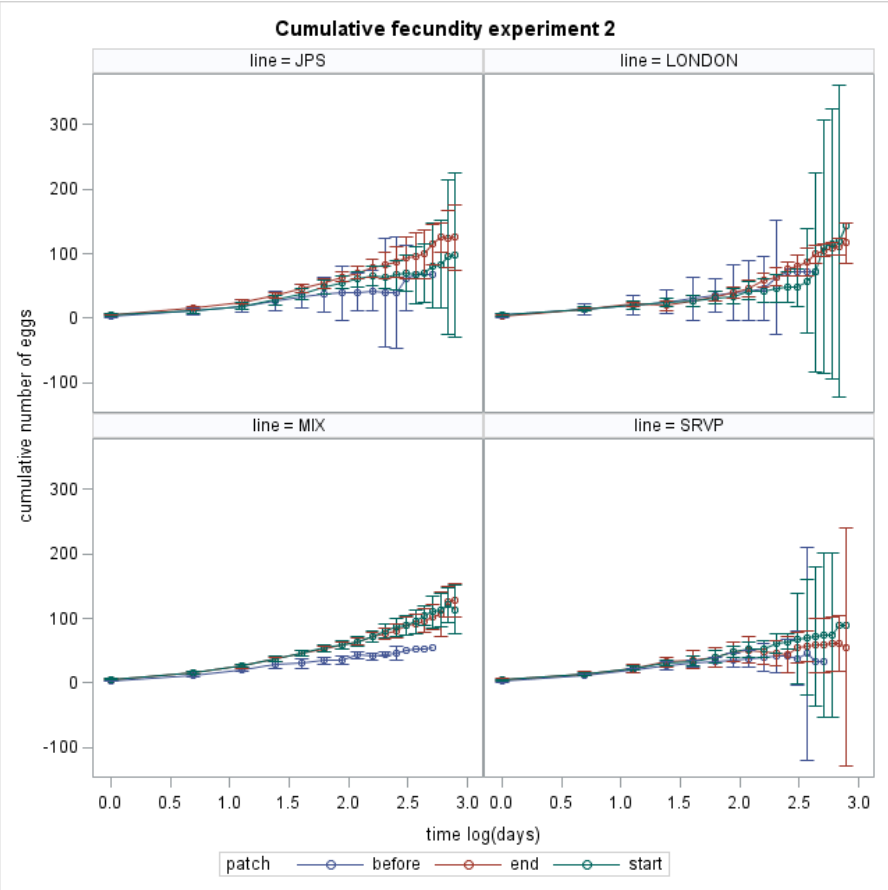
Experiment 2	ECO-EVO2	ECO
Start-end	Slope: $F_{1,1131}$=0.13; p=0.7194 Intercept: $F_{1,10.1}$=0.14; p=0.7116	Slope: $F_{1,1121}$=0.02; p=0.9023 Intercept: $F_{1,11.3}$=0.190; p=0.6704
End-stock	Slope: **$F_{1,700}$=92.19; p<0.0001** Intercept: $F_{1,102}$=0.94; p=0.3336	Slope: **$F_{1,737}$=47.18; p<0.0001** Intercept: $F_{1,5.11}$=1.42; p=0.2856
Start-stock	Slope: **$F_{1,672}$=69.45; p<0.0001** Intercept: $F_{1,172}$=1.66; p=0.1998	Slope: **$F_{1,765}$=32.89; p<0.0001** Intercept: **$F_{1,7.63}$=6.41; p=0.0365**

Appendix 1. 95: Statistical output of cumulative fecundity for experiment 2

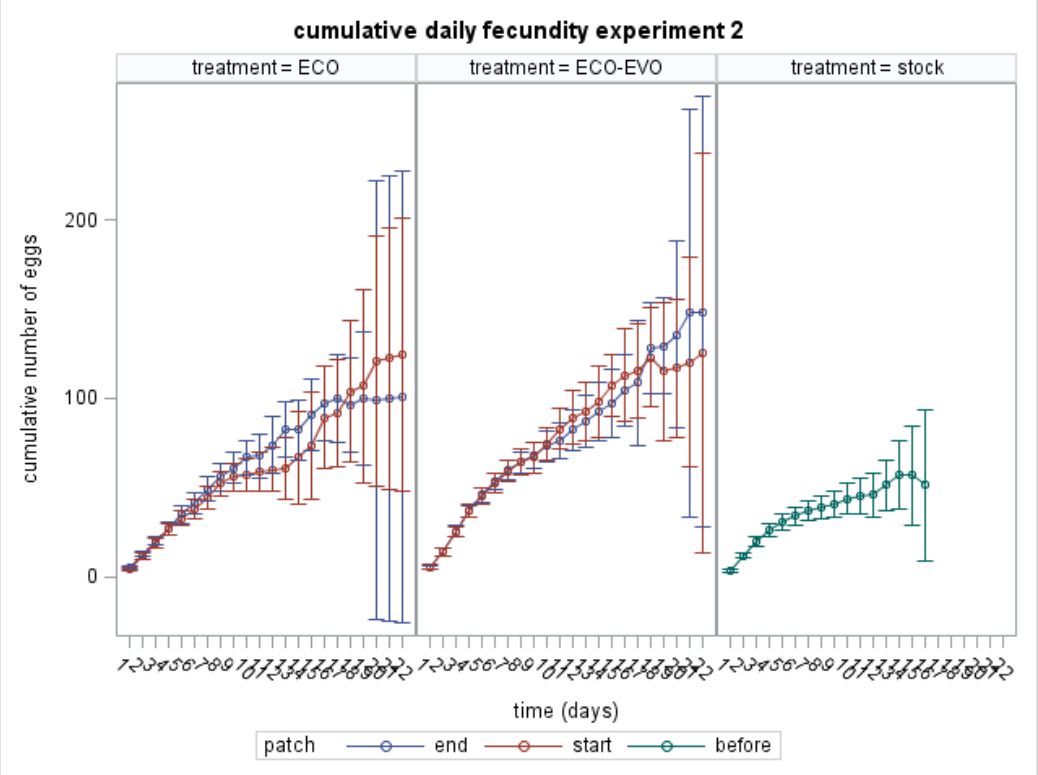
Even though there were no differences in cumulative fecundity between populations from the core or from the dispersal front in either of both experiments, there was however a consistent trend to a faster increase in cumulative fecundity compared to the stock population. Differences were significant for all treatments except the evolutionary constrained one ffrom experiment 1 (RFS). Likely (as suggested before by mean daily fecundity), oviposition is at least partially regulated by plasticity, and the experimental setup appears to increase early reproduction. The lack of responses in the RFS treatment, may stem from the fact that individuals were replaced up to two weeks prior to collection. The two week period may have been insufficient to induce the same responses as in the other treatments.



Appendix 1. 96: Cumulative fecundity by treatment and patch for experiment 1



Appendix 1. 97: Cumulative fecundity by line and patch for experiment 2



Appendix 1. 98: Cumulative fecundity by treatment and patch for experiment 2

Longevity

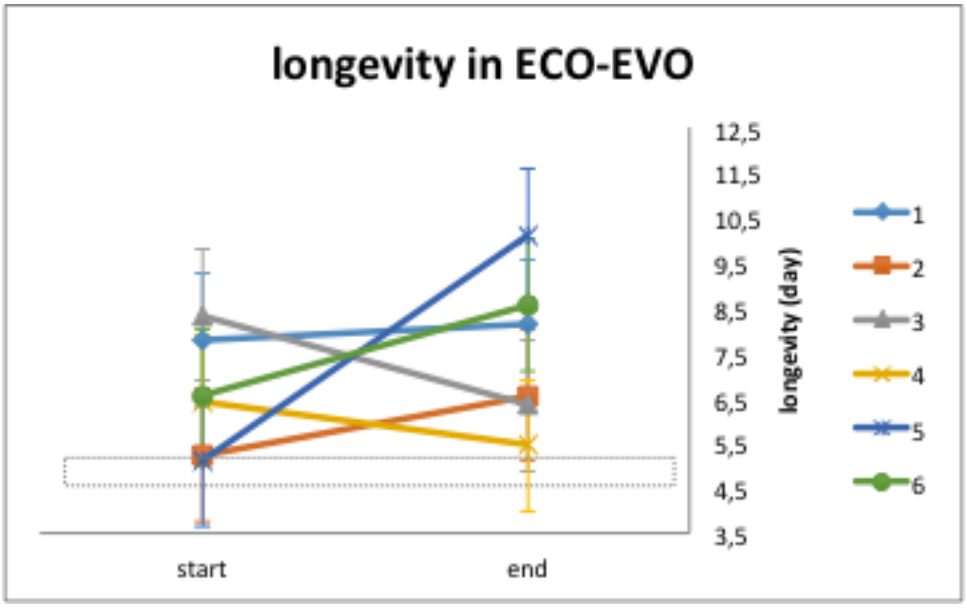
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,10}$=1.15; p=0.3091	$F_{1,4}$=0.13; p=0.7382
End-stock	$F_{1,2.11}$=3.17; p=0.2105	**$F_{1,15.2}$=8.14; p=0.0119**
Start-stock	$F_{1,1.62}$=2.61; p=0.2749	**$F_{1,13}$=17.36; p=0.0011**

Appendix 1. 99: Statistical output of longevity for experiment 1

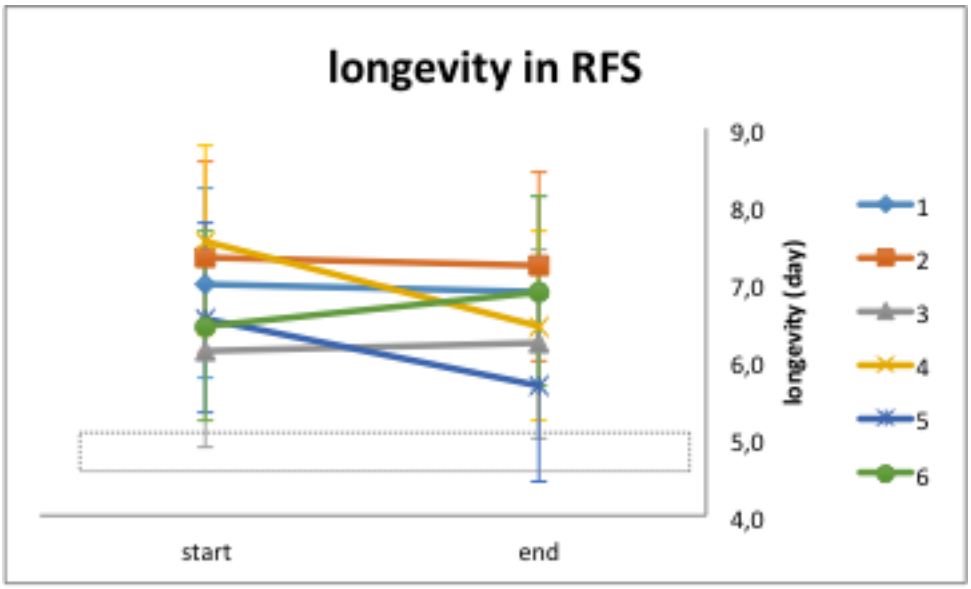
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,5.33}$=0.16; p=0.7039	$F_{1,103}$=0.54; p=0.4635
End-stock	$F_{1,5.5}$=0.13 p=0.7316	$F_{1,68}$ =0.48; p=0.4900
Start-stock	$F_{1,4.39}$=0.00; p=0.9624	$F_{1,5.37}$=0.01; p=0.9119

Appendix 1. 100: Statistical output of longevity for experiment 2

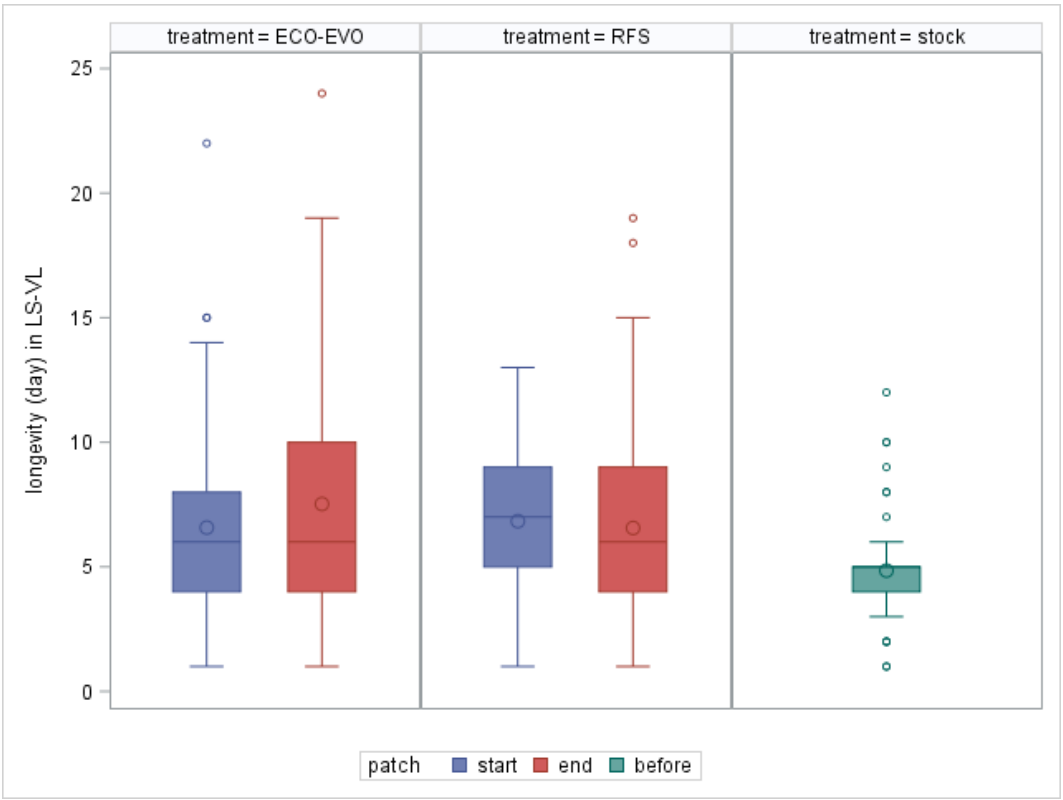
Longevity differs only for both core and dispersal front populations from the stock in the evolutionary constrained (RFS) treatment of experiment 1. This trend is however not consistent across experiments.



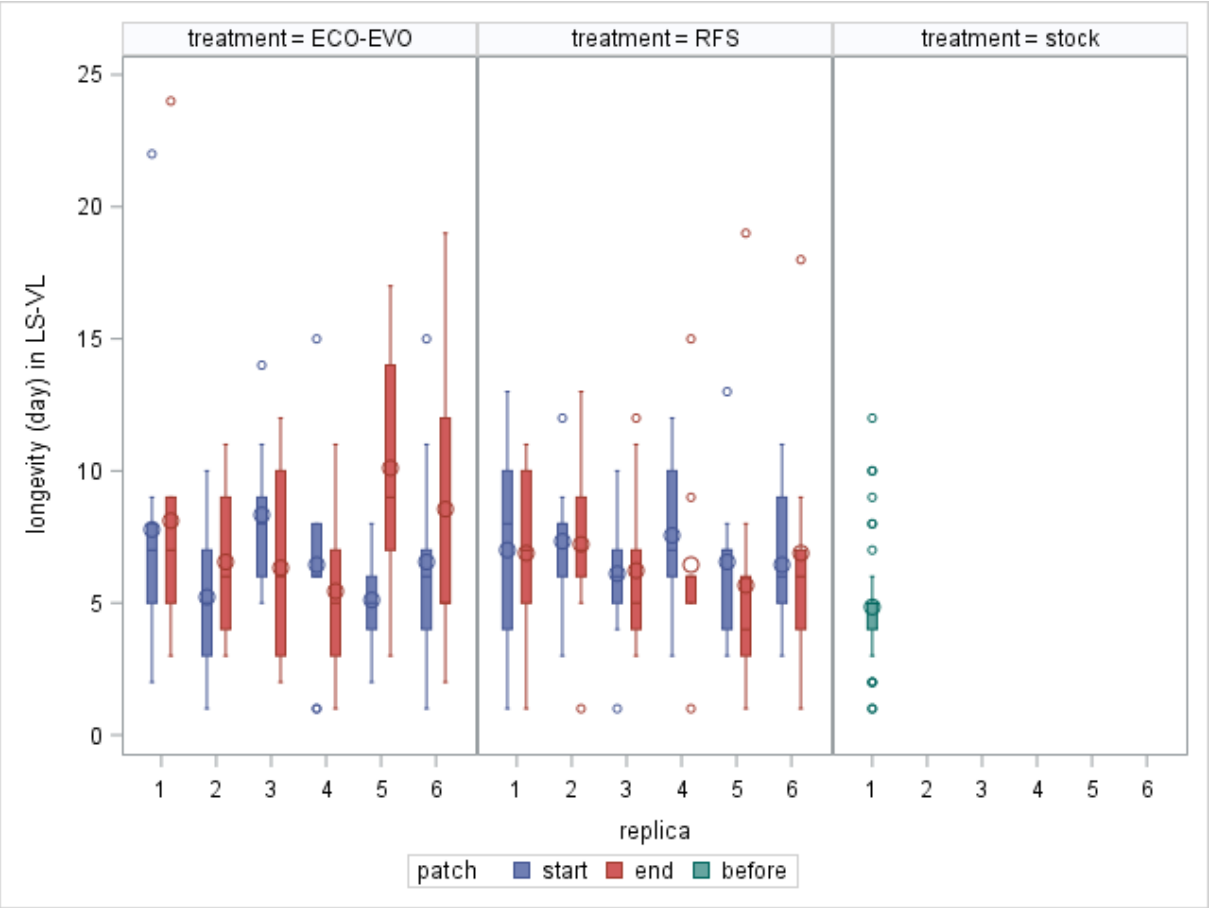
Appendix 1. 101: Effect sizes by replica for longevity of the ECO-EVO1 treatment of experiment 1



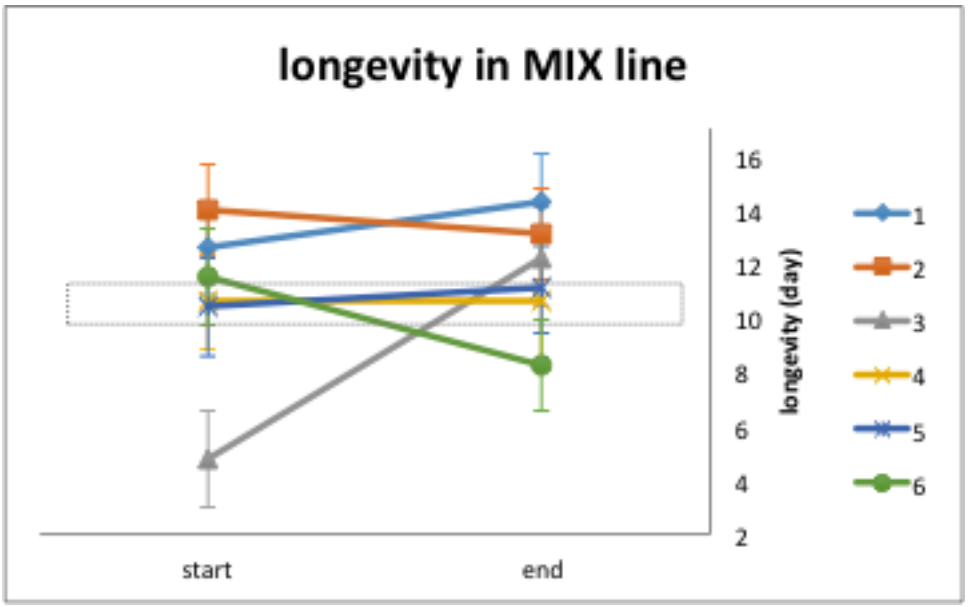
Appendix 1. 102: Effect sizes by replica for longevity of the RFS treatment of experiment 1



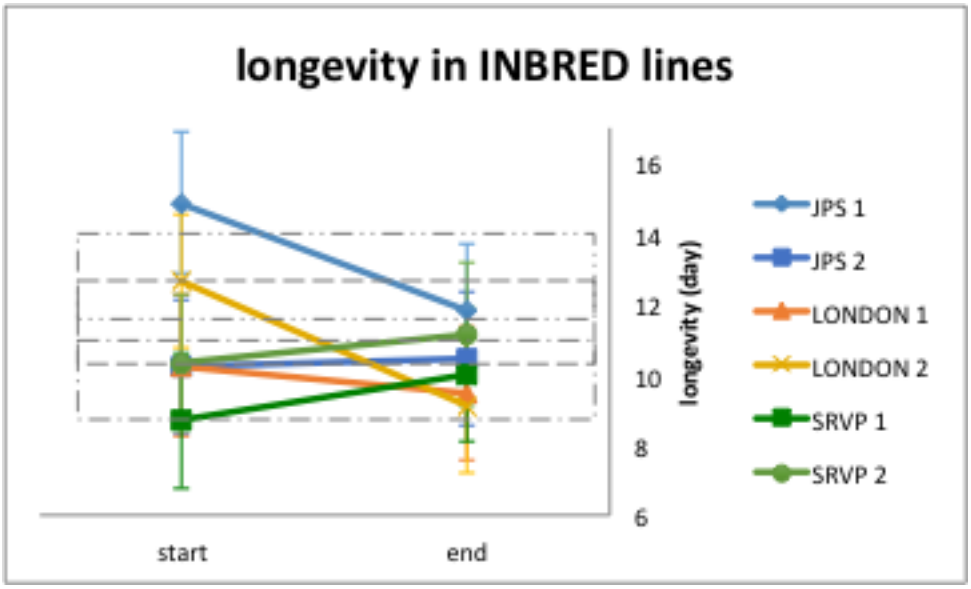
Appendix 1. 103: Longevity by treatment and patch for experiment 1



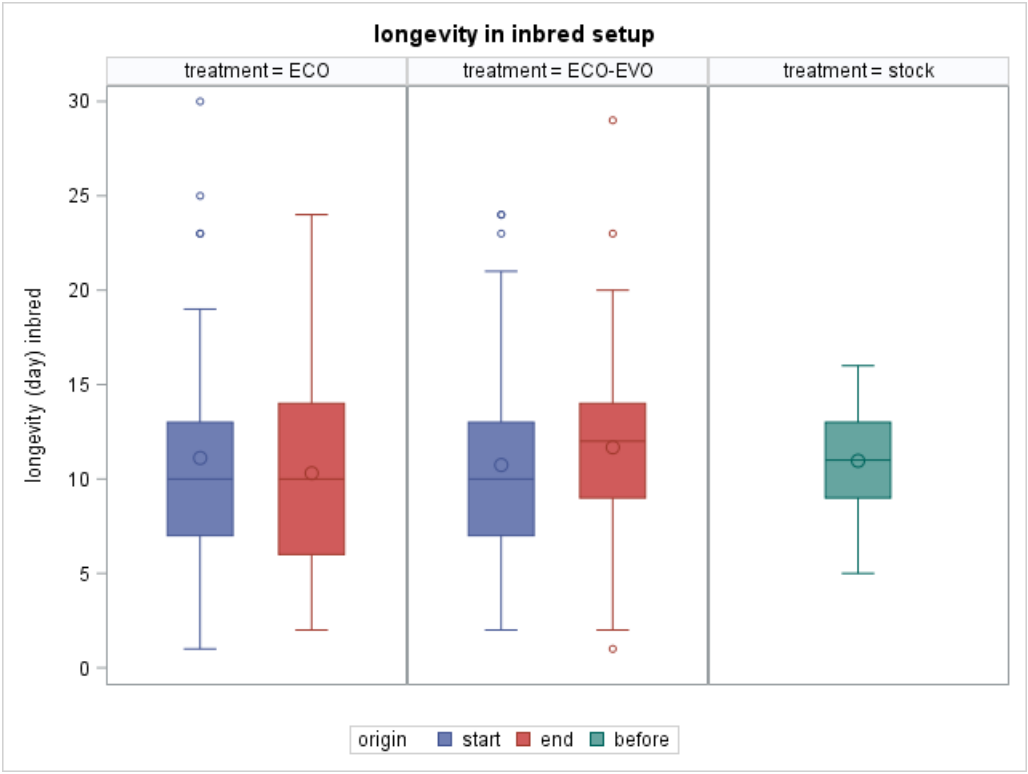
Appendix 1. 104: Longevity by replica and patch for experiment 1



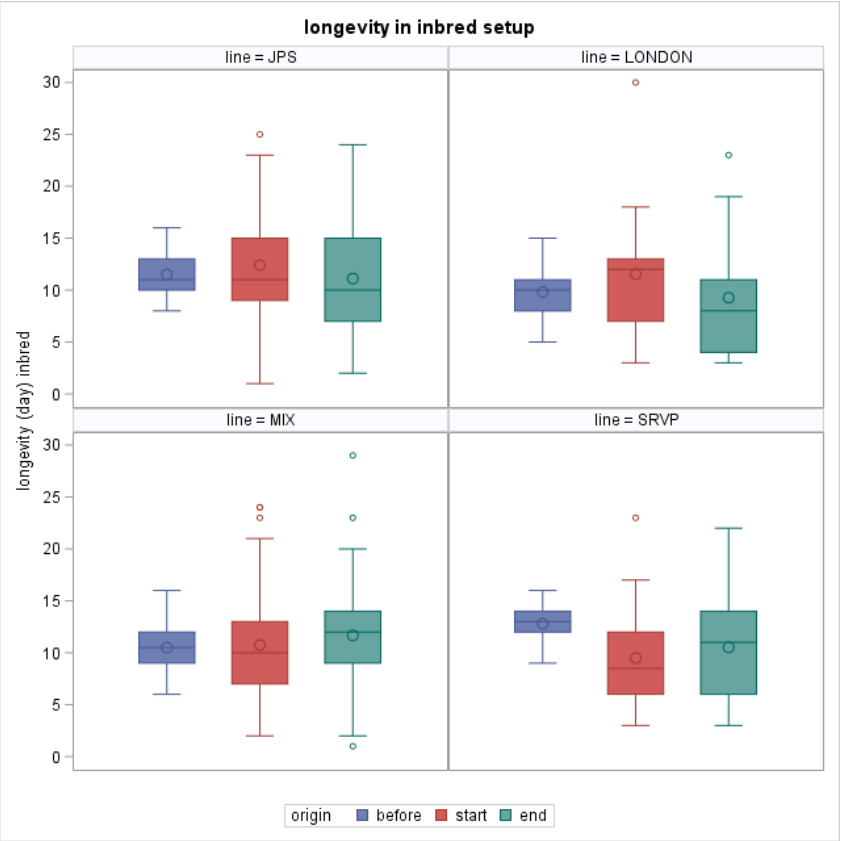
Appendix 1. 105: Effect sizes by replica for longevity of the ECO-EVO2 treatment of experiment 2



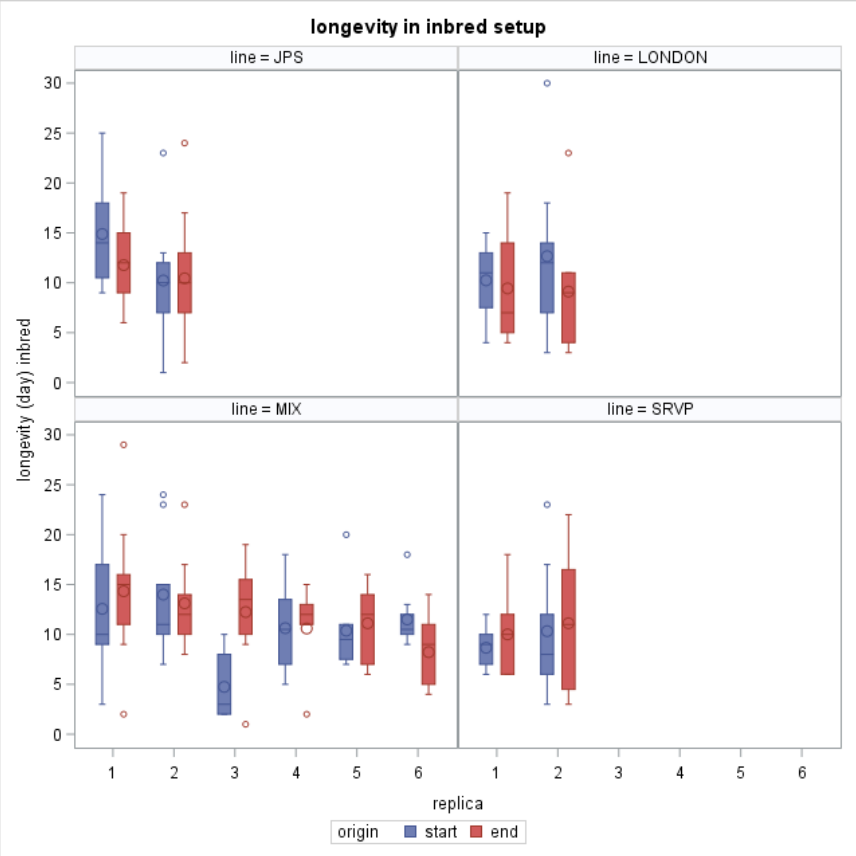
Appendix 1. 106: Effect sizes by replica for longevity of the ECO-EVO2 treatment of experiment 2



Appendix 1. 107: Longevity by treatment and patch for experiment 2



Appendix 1. 108: Longevity by line and patch for experiment 2



Appendix 1. 109: Longevity by replica and patch for experiment 2

Leaf consumption

Experiment 1: 3 days	ECO-EVO1	RFS
Start-end	$F_{1,3.88}$=0.49; p=0.5244	$F_{1,67.7}$=0.82; p=0.3689
End-stock	$F_{1,2.17}$=0.42 p=0.5808	$F_{1,2.51}$ =0.44; p=0.5641
Start-stock	$F_{1,5.39}$=3.67; p=0.1095	$F_{1,2.37}$=1.72; p=0.3023

Appendix 1. 110: Statistical output of leaf consumption after 3 days for experiment 1

Experiment 1: 5 days	ECO-EVO1	RFS
Start-end	$F_{1,49.9}$=1.72; p=0.1960	$F_{1,1.66}$=0.03; p=0.8721
End-stock	$F_{1,2.06}$=0.34 p=0.6162	$F_{1,2.17}$ =0.11; p=0.7722
Start-stock	$F_{1,33}$=0.00; p=0.9496	$F_{1,1.96}$=0.28; p=0.6526

Appendix 1. 111: : Statistical output of leaf consumption after 5 days for experiment 1

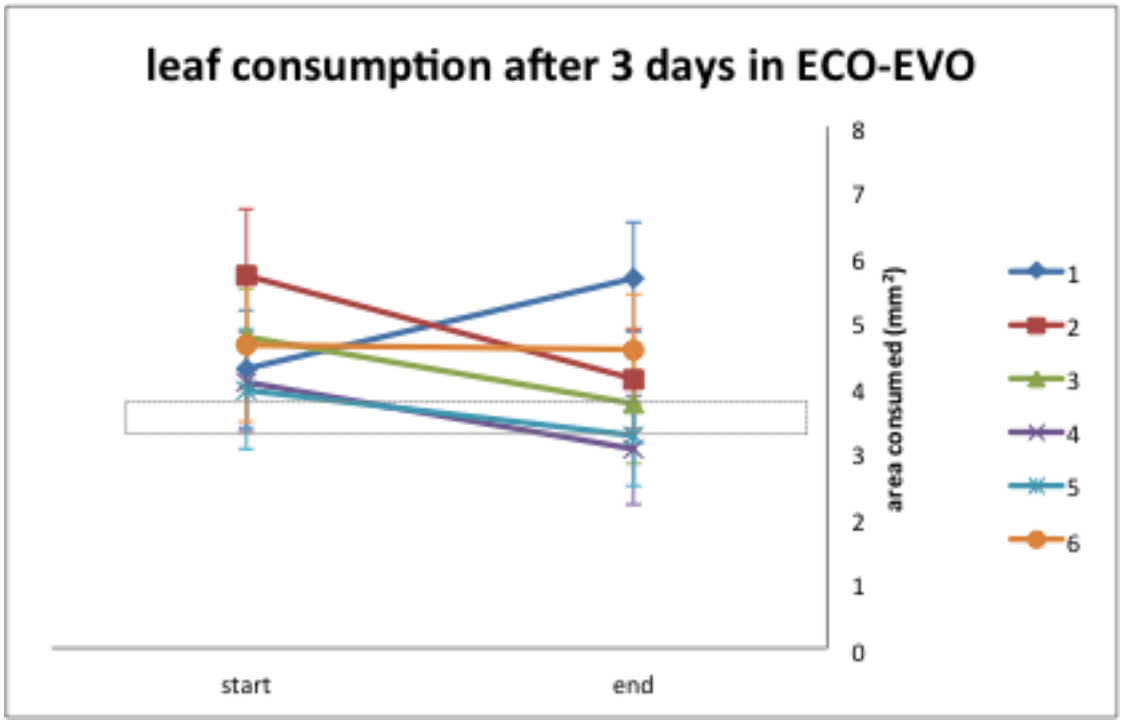
Experiment 2: 3 days	ECO-EVO2	ECO
Start-end	**$F_{1,64}$=4.11; p=0.0469**	$F_{1,10.2}$=0.03; p=0.8709
End-stock	$F_{1,31}$=2.11 p=0.1566	$F_{1,6.25}$=2.78; p=0.1444
Start-stock	$F_{1,32}$=2.46; p=0.1269	$F_{1,3.8}$=3.64; p=0.1327

Appendix 1. 112: Statistical output of leaf consumption after 3 days for experiment 2

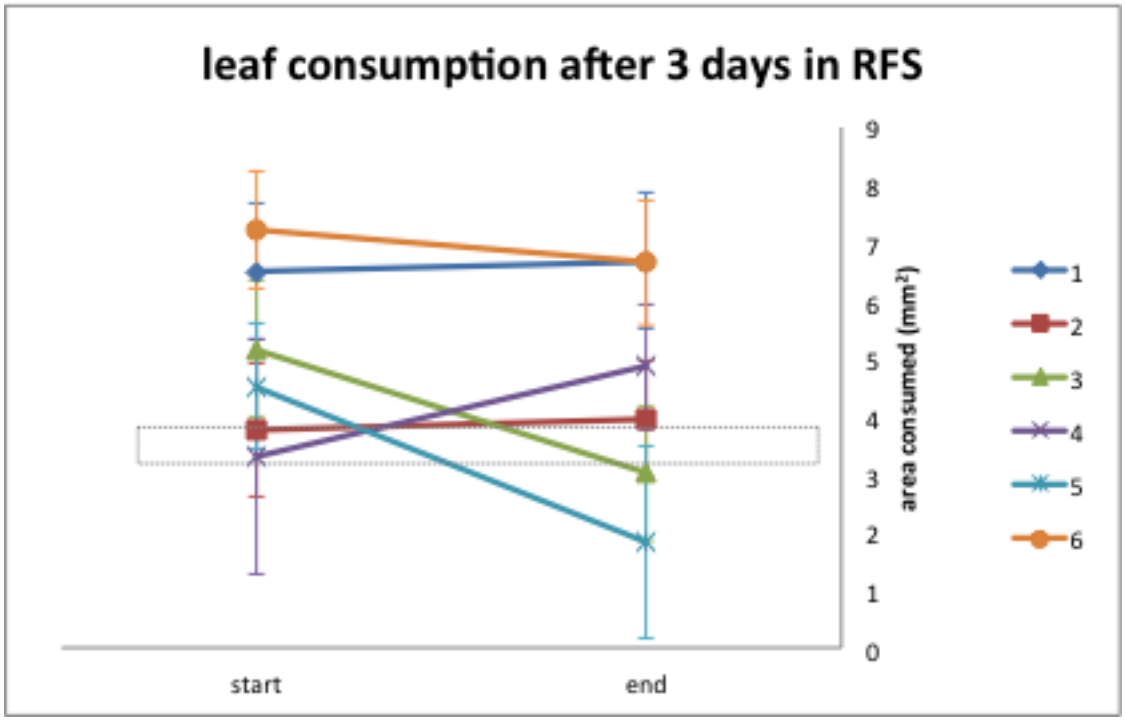
Experiment 2: 5 days	ECO-EVO2	ECO
Start-end	$F_{1,3.95}$=1.03; p=0.3685	$F_{1,8.75}$=1.79; p=0.2152
End-stock	$F_{1,16.8}$=3.14 p=0.0947	$F_{1,5.4}$=0.41; p=0.5460
Start-stock	$F_{1,24.9}$=1.98; p=0.1717	F $F_{1,3.79}$=0.00; p=0.9474

Appendix 1. 113: Statistical output of leaf consumption after 5 days for experiment 2

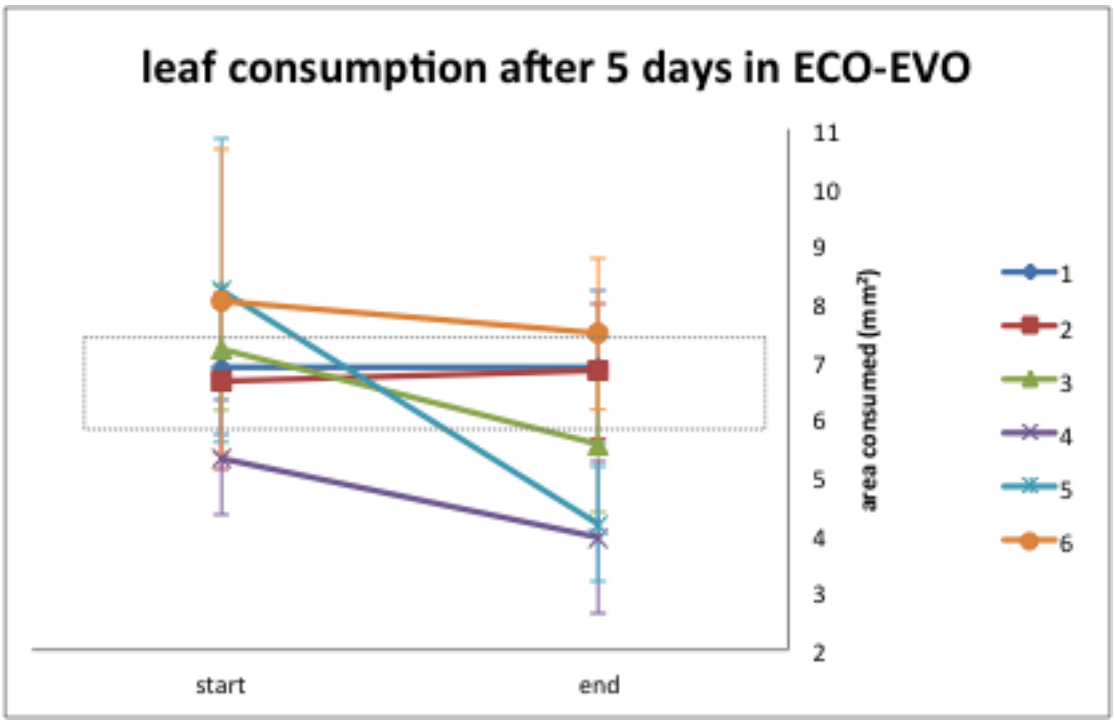
Leaf consumption (both after 3 days and 5 days) does not differ significantly for either the core populations or the populations from the dispersal front for any of the treatments.



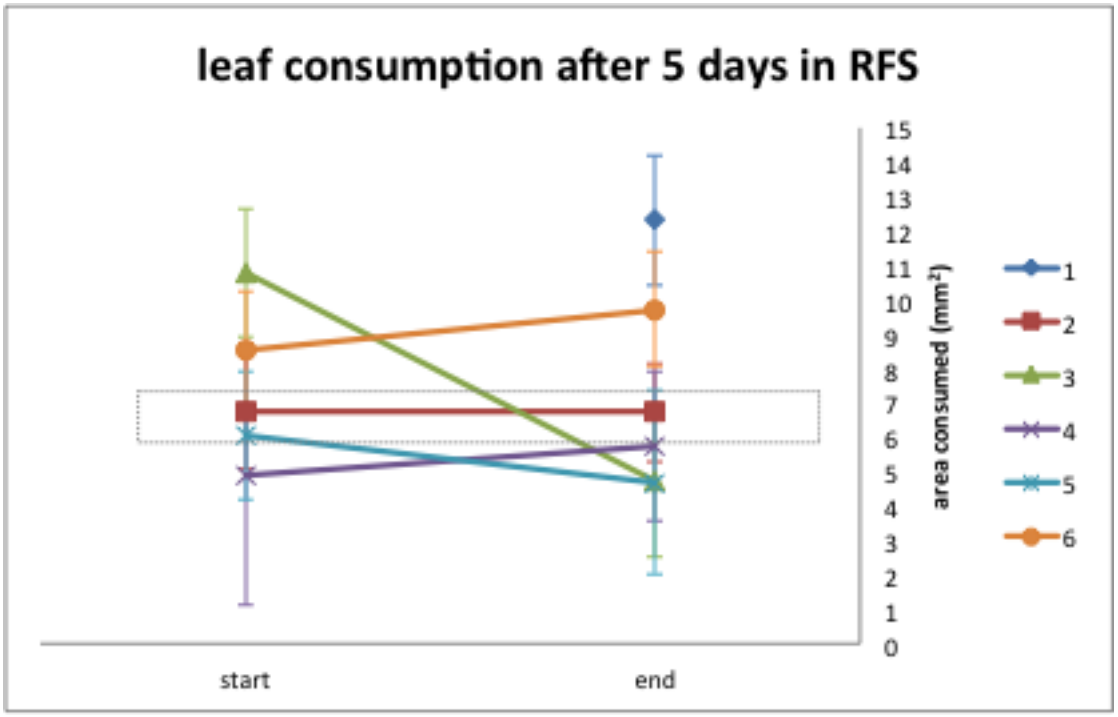
Appendix 1. 114: Effect sizes by replica of leaf consumption after 3 days for the ECO-EVO1 treatment of experiment 1



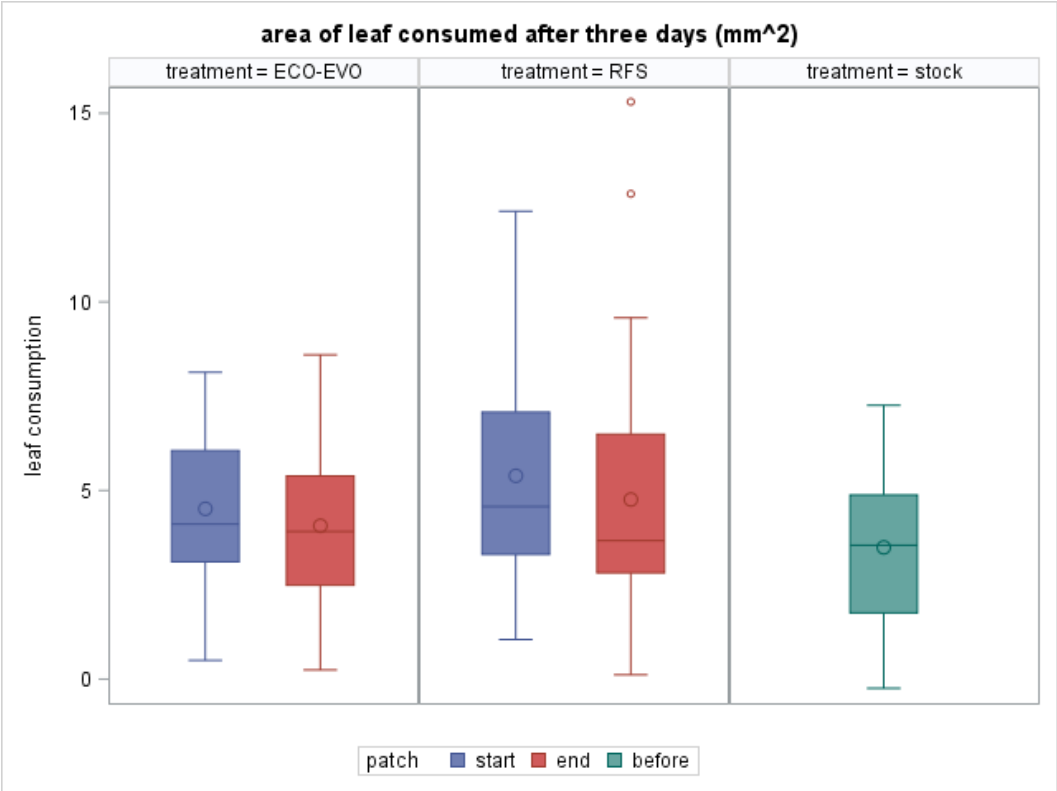
Appendix 1. 115: Effect sizes by replica of leaf consumption after 3 days for the RFS treatment of experiment 1



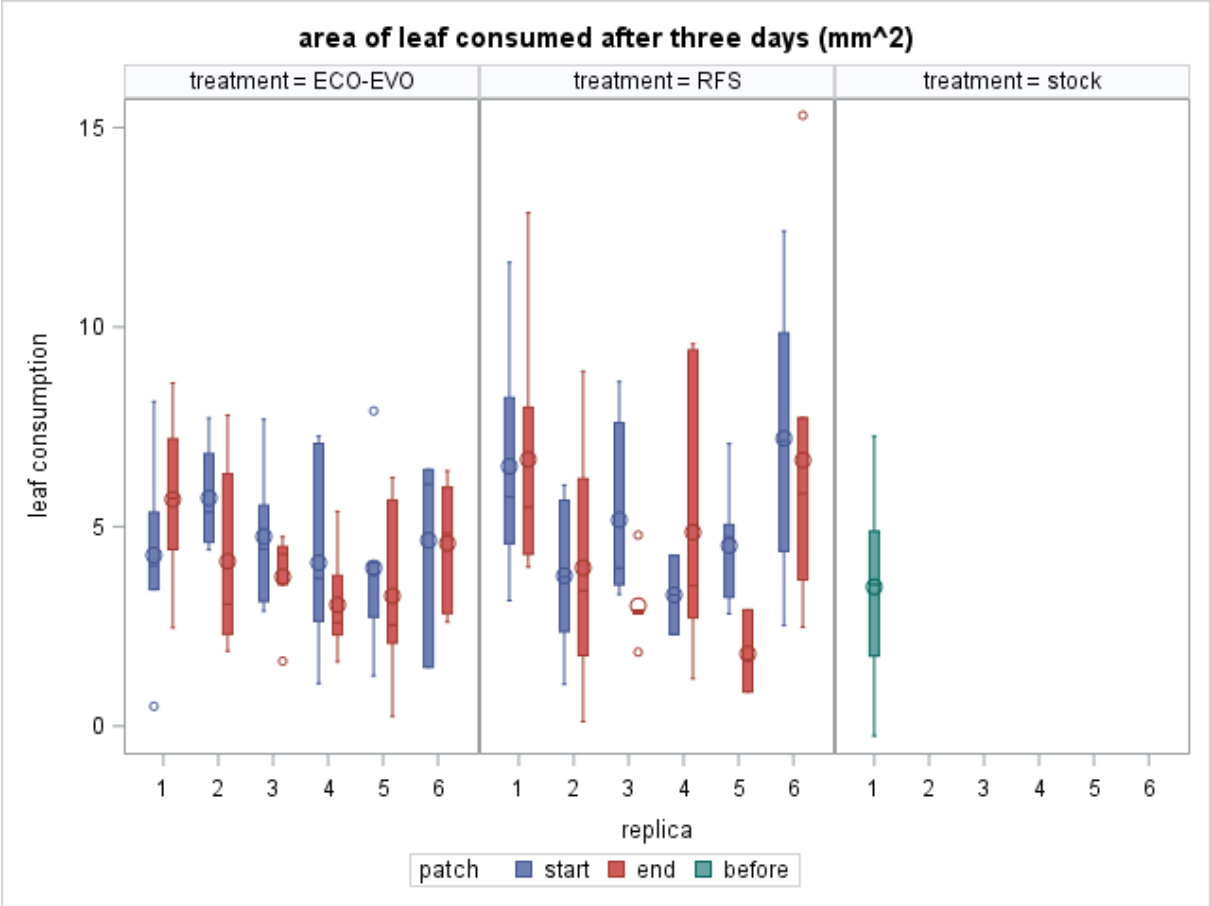
Appendix 1. 116: Effect sizes by replica of leaf consumption after 5 days for the ECO-EVO1 treatment of experiment 1



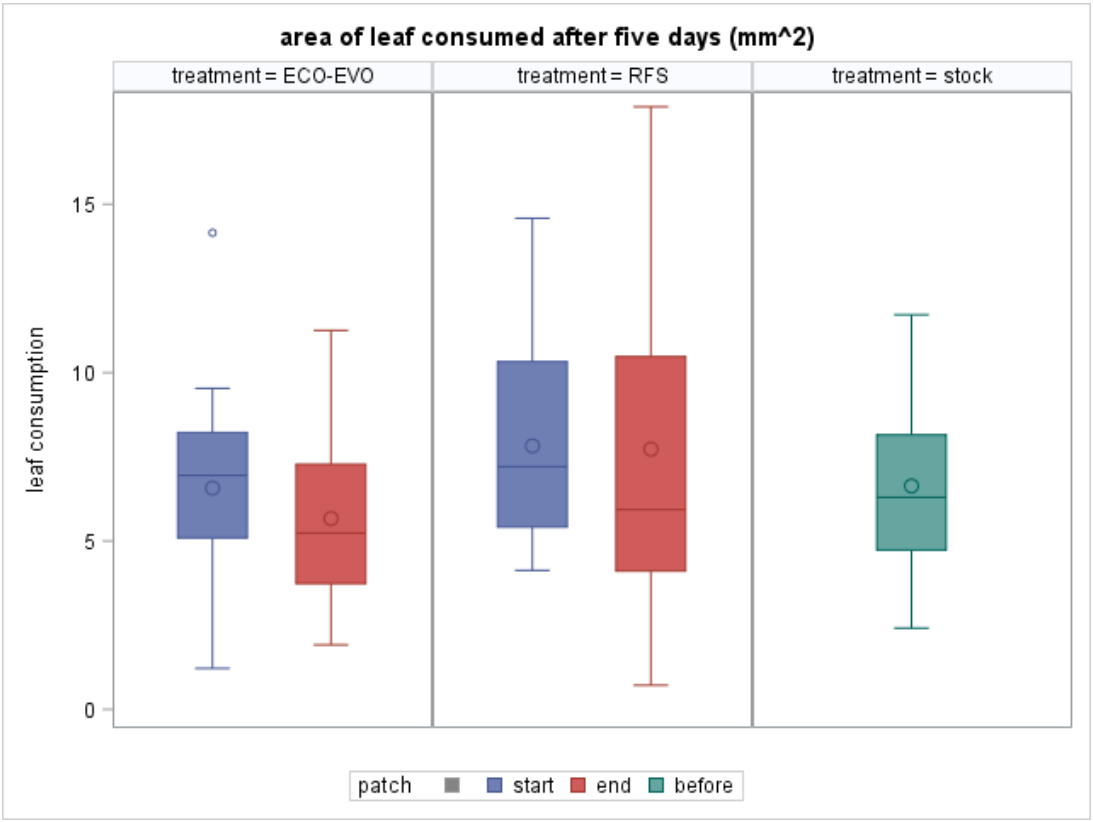
Appendix 1. 117: Effect sizes by replica of leaf consumption after 5 days for the RFS treatment of experiment 1



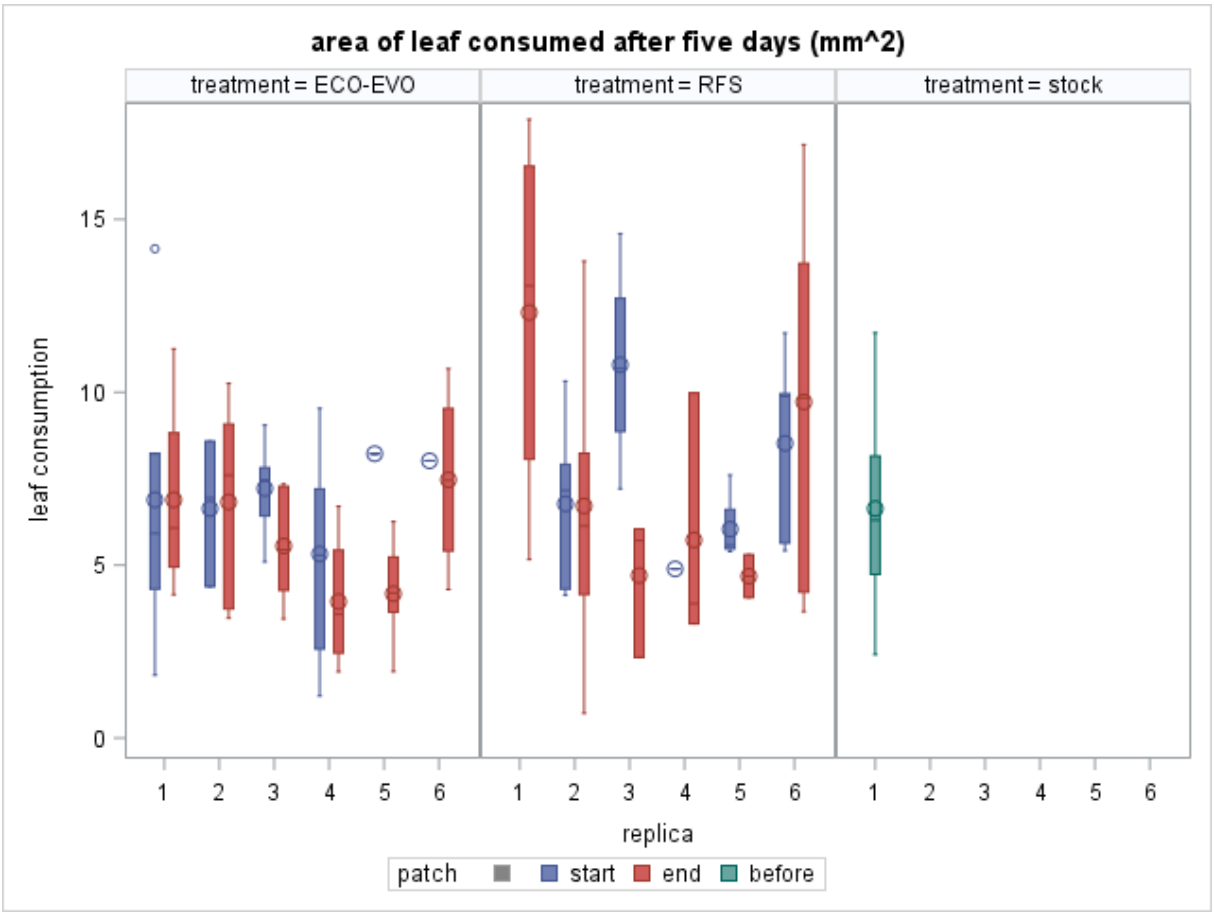
Appendix 1. 118: Leaf consumption after 3 days by treatment and patch for experiment 1



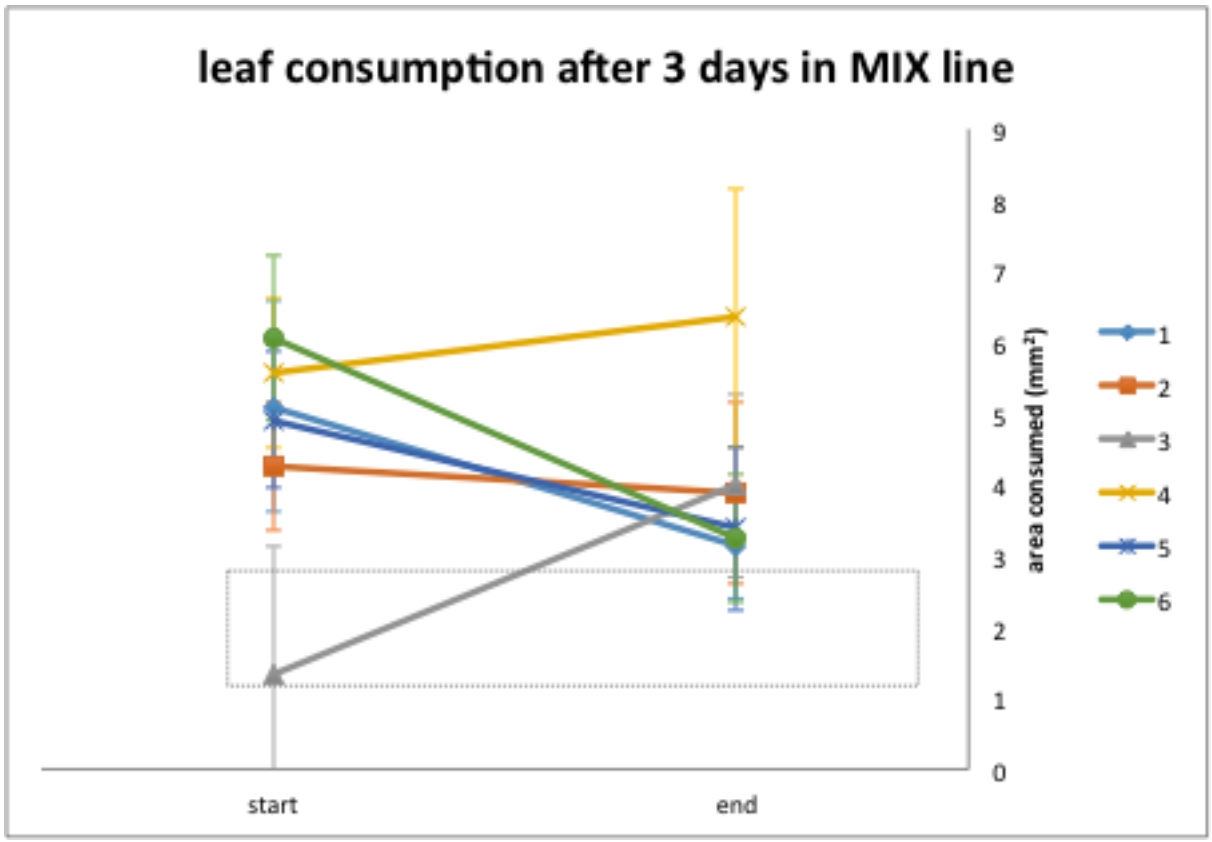
Appendix 1. 119: Leaf consumption after 3 days by replica and patch for experiment 1



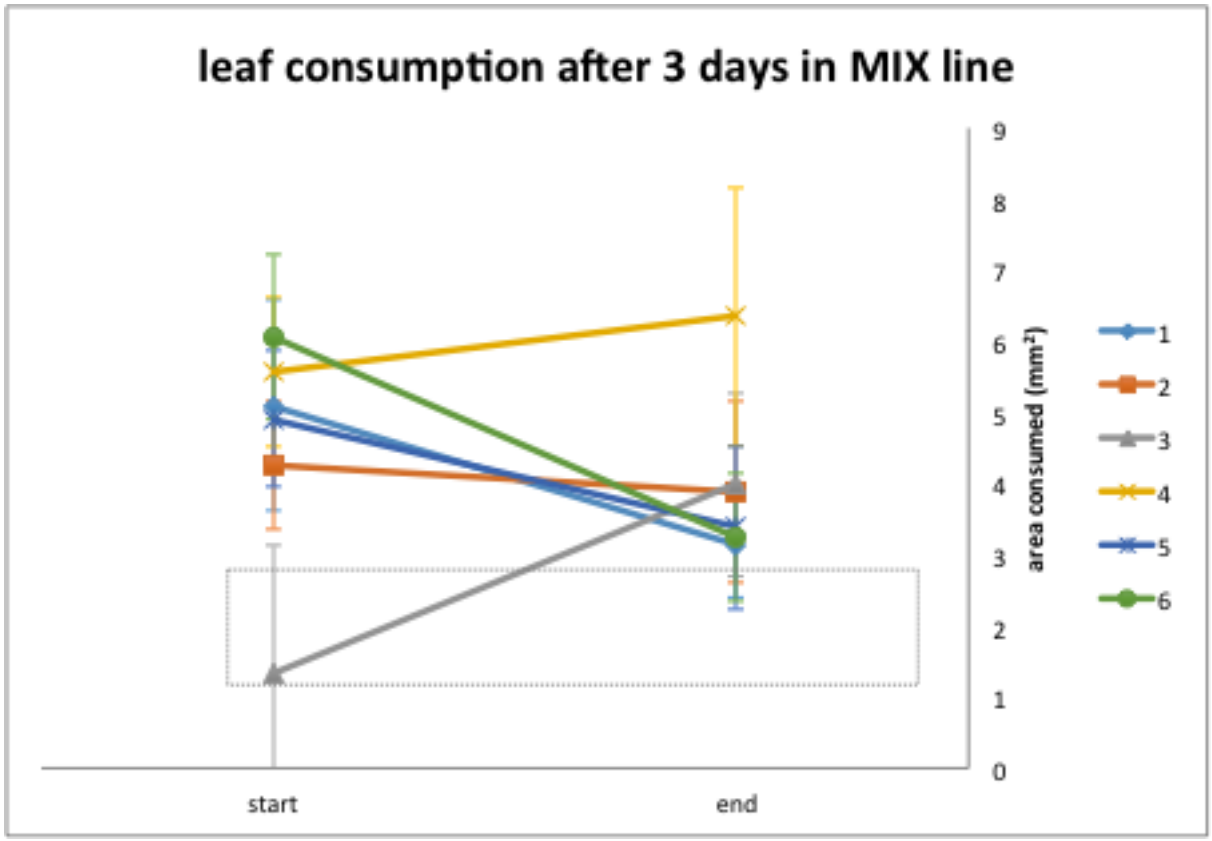
Appendix 1. 120: Leaf consumption after 5 days by treatment and patch for experiment



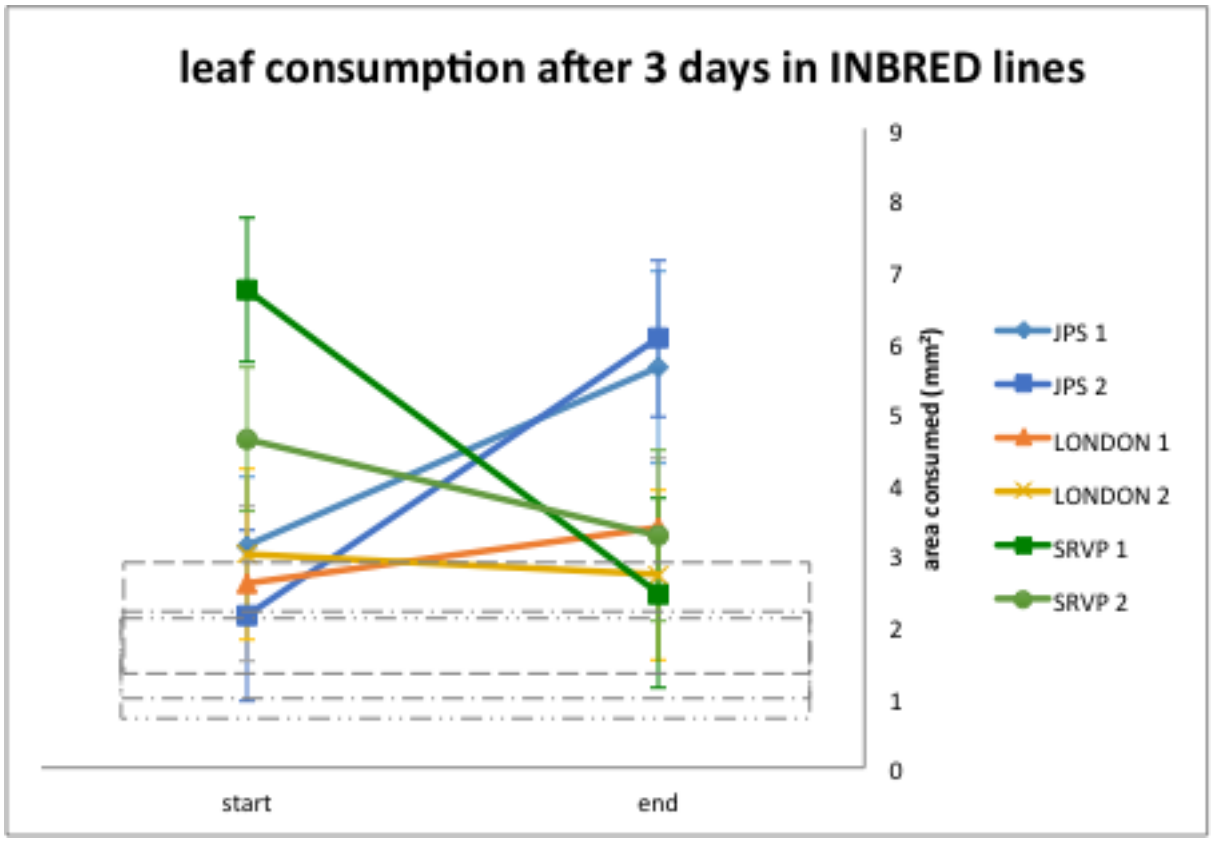
Appendix 1. 121: Leaf consumption after 5 days by replica and patch for experiment 1



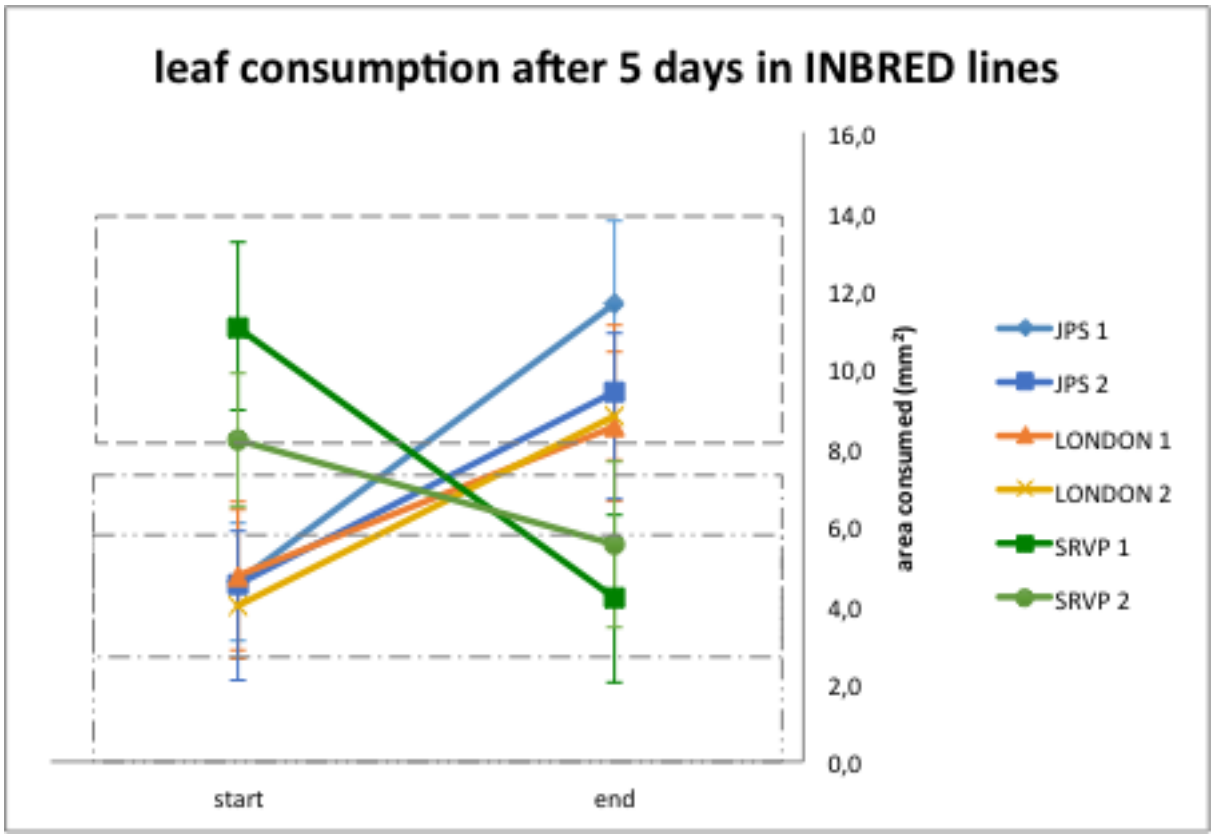
Appendix 1. 122: Effect sizes by replica of leaf consumption after 3 days for the ECO-EVO2 treatment of experiment 2



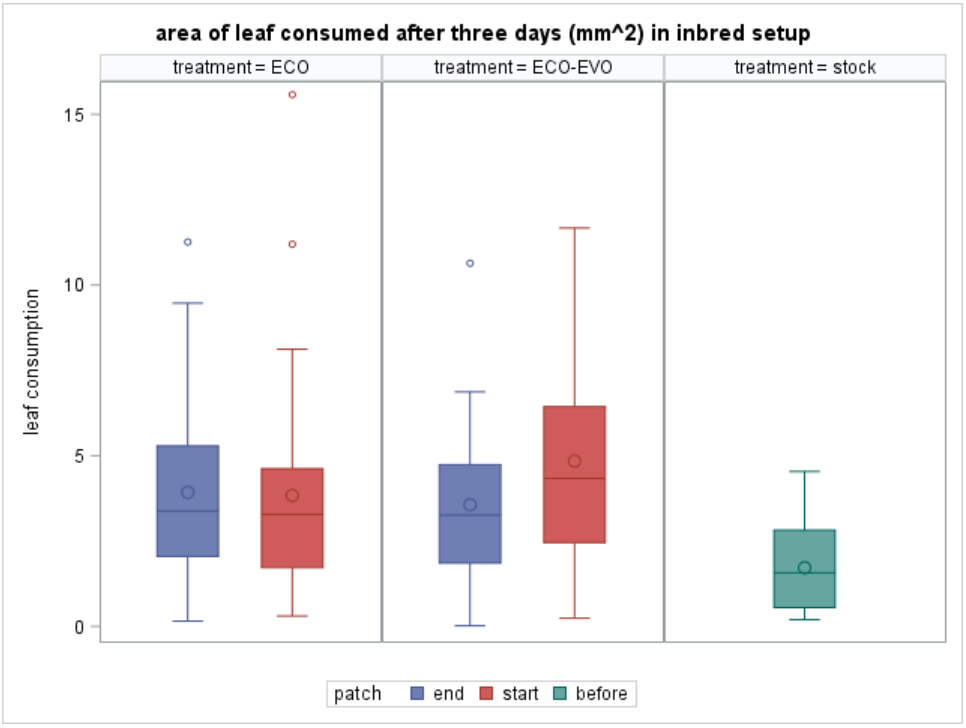
Appendix 1. 123: Effect sizes by replica of leaf consumption after 5 days for the ECO-EVO2 treatment of experiment 2



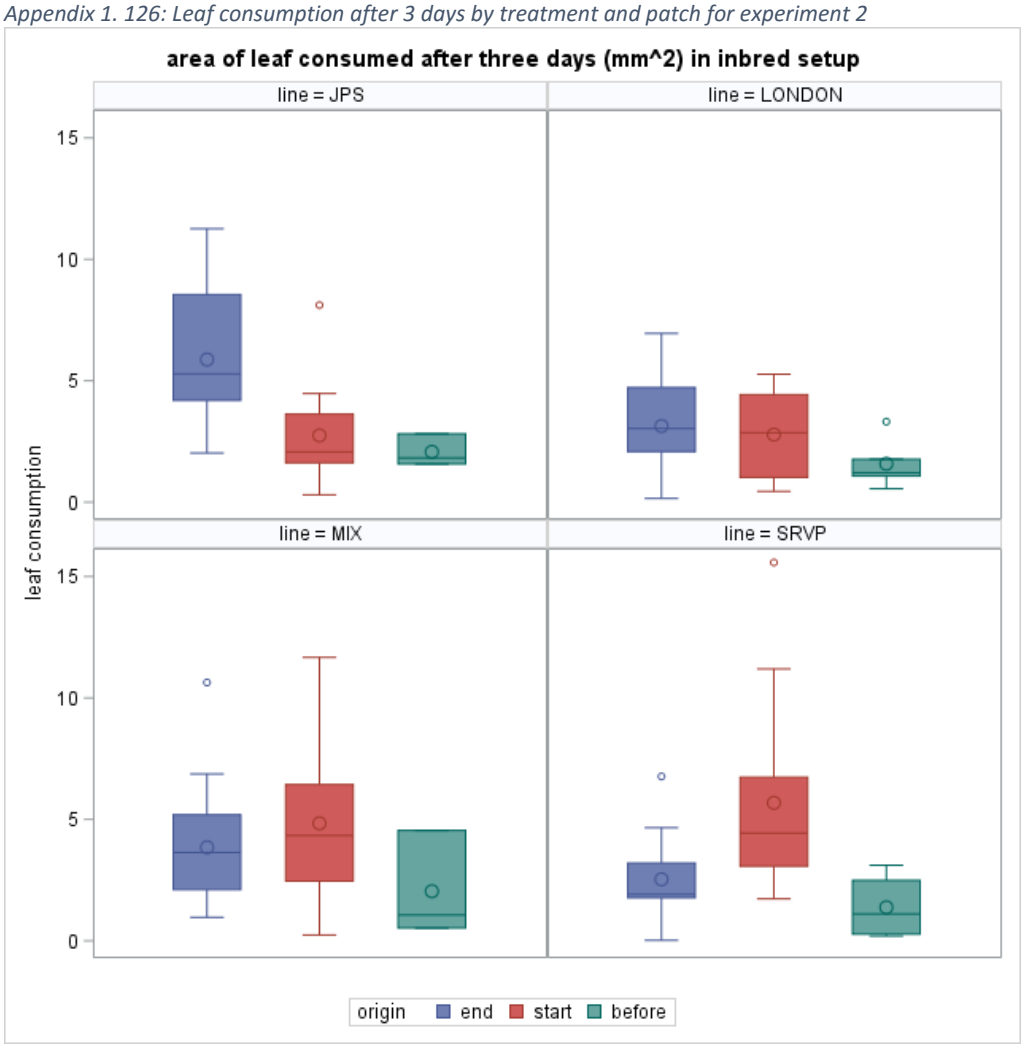
Appendix 1. 124: Effect sizes by replica of leaf consumption after 3 days for the ECO treatment of experiment 2



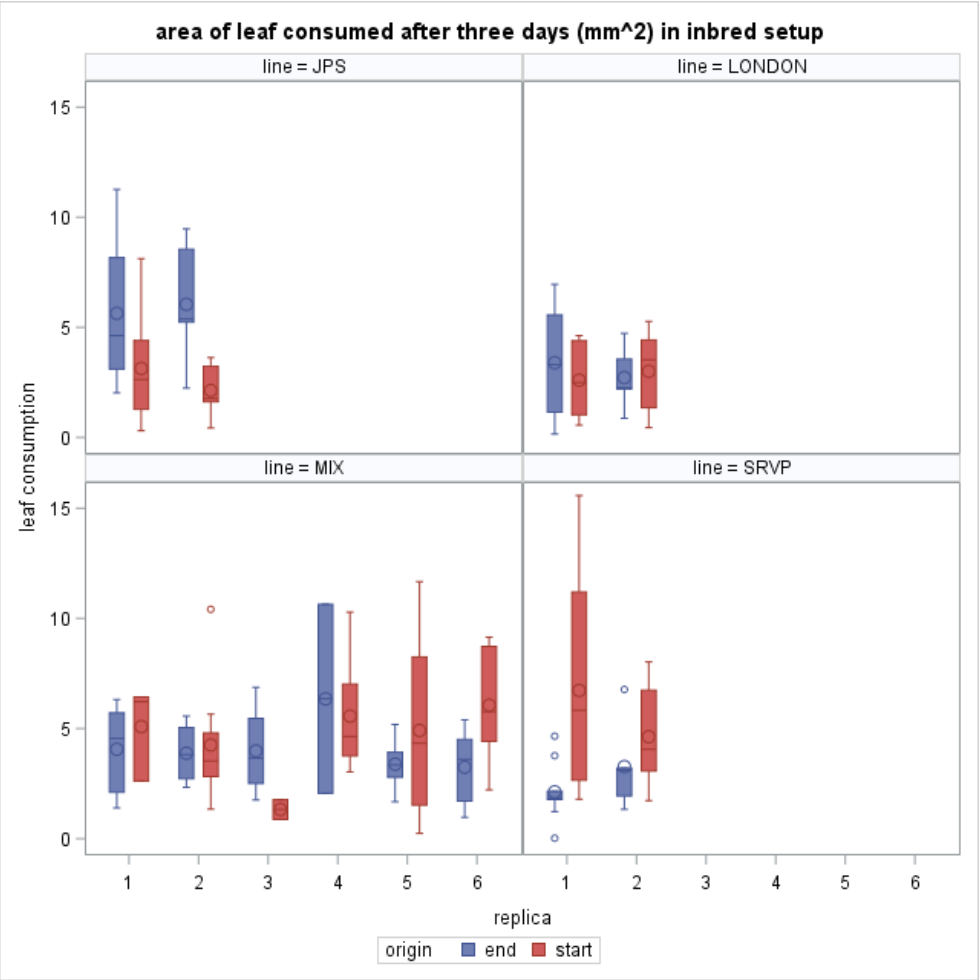
Appendix 1. 125: Effect sizes by replica of leaf consumption after 5 days for the ECO treatment of experiment 2



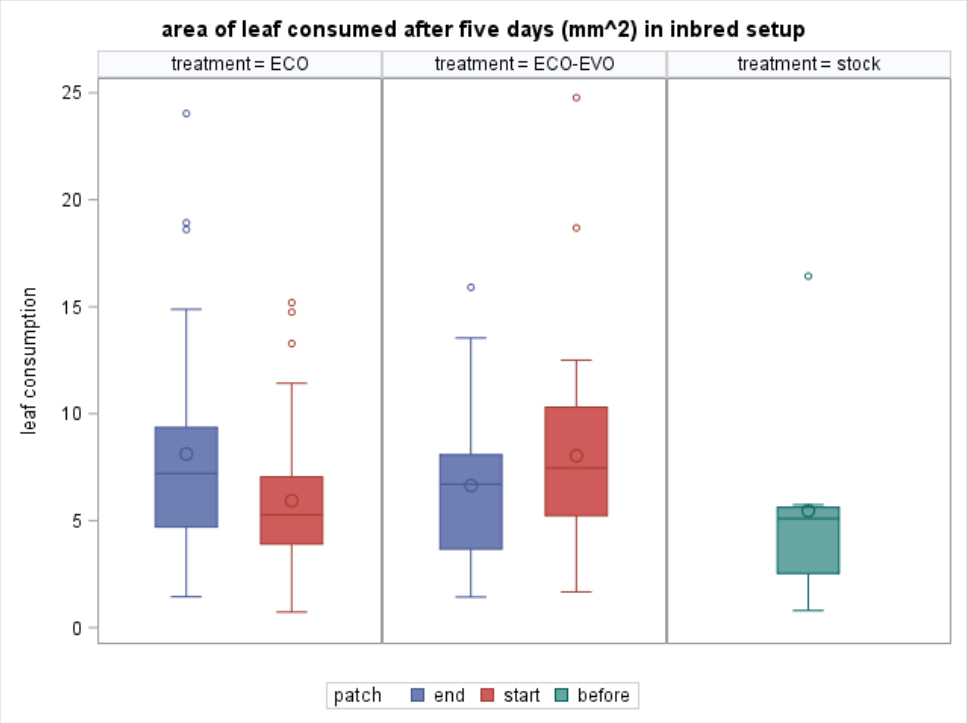
Appendix 1. 126: Leaf consumption after 3 days by treatment and patch for experiment 2



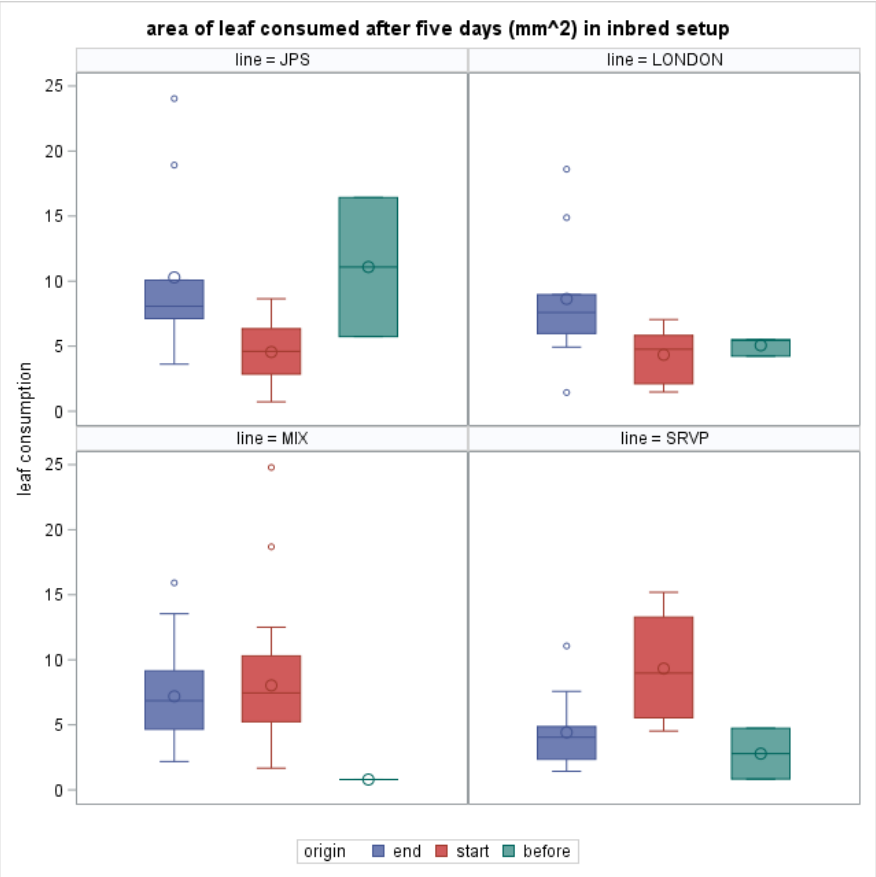
Appendix 1. 127: Leaf consumption after 3 days by line and patch for experiment 2



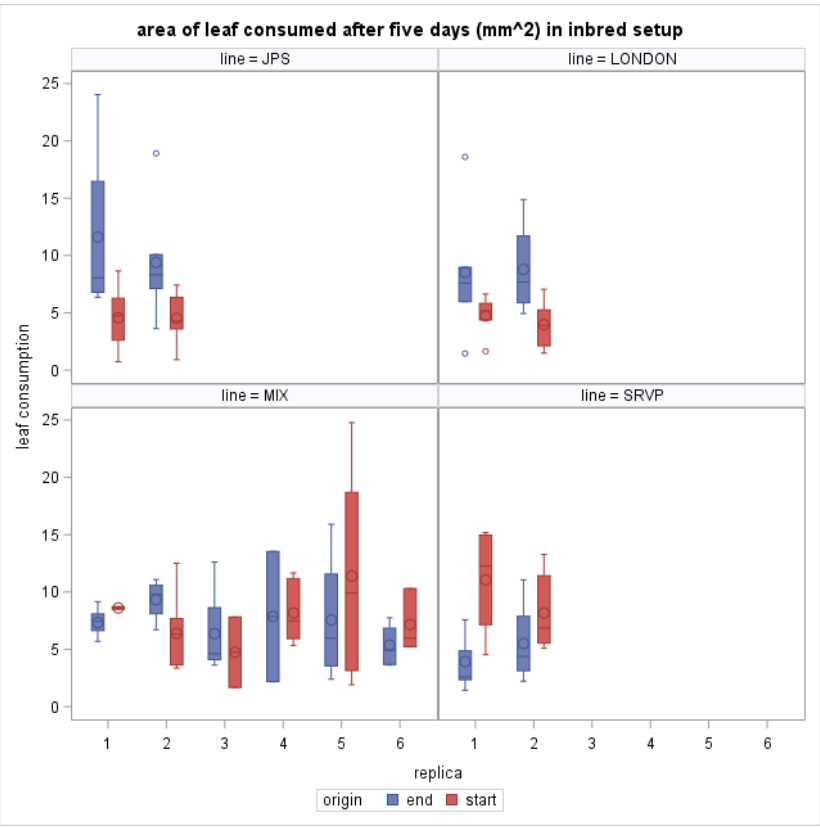
Appendix 1. 128: Leaf consumption after 3 days by replica and patch for experiment 2



Appendix 1. 129: Leaf consumption after 5 days by treatment and patch for experiment 2



Appendix 1. 130: Leaf consumption after 5 days by line and patch for experiment 2



Appendix 1. 131: Leaf consumption by replica and patch for experiment 2

Ambulatory dispersal: mean distance moved at day 4

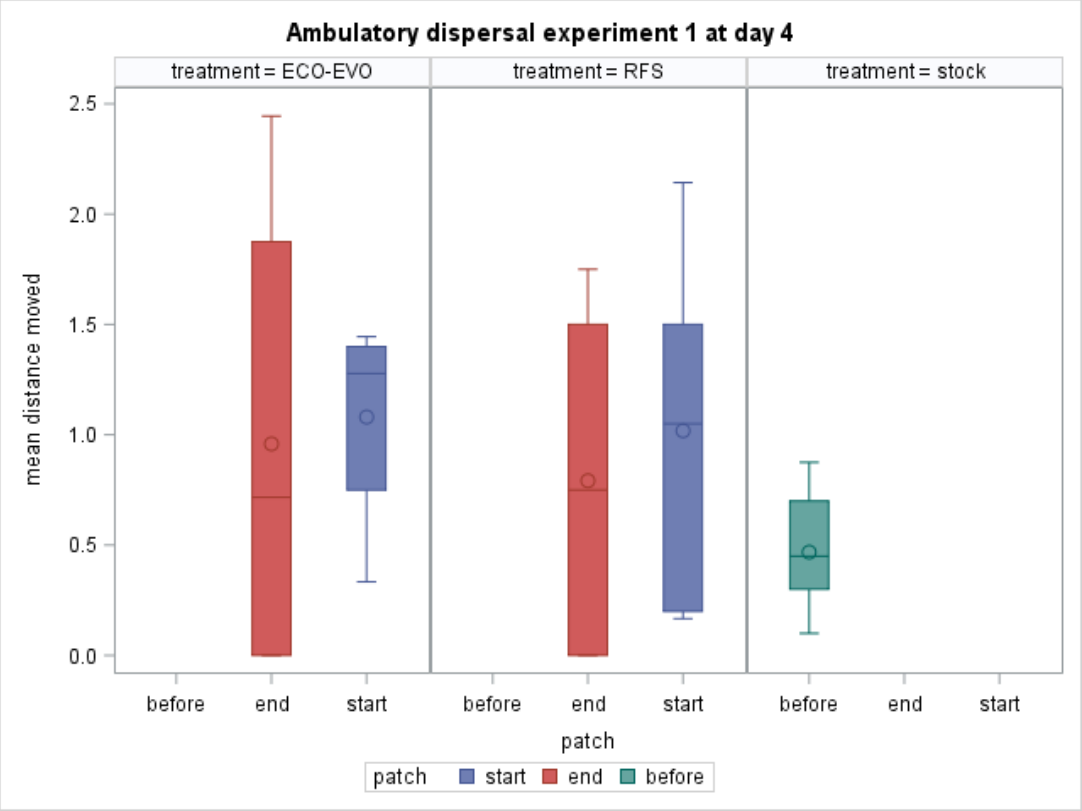
Experiment 1	ECO-EVO1	RFS
Start-end	$F_{1,10}$=0.07; p=0.7956	$F_{1,10}$=0.27; p=0.6145
End-stock	$F_{1,14}$=2.15p=0.1647	$F_{1,14}$ =1.62; p=0.2236
Start-stock	**$F_{1,14}$=12.44; p=0.0034**	$F_{1,14}$ =4.55; p=0.0511

Appendix 1. 132: Statistical output of mean distance moved at the 4th day for experiment 1

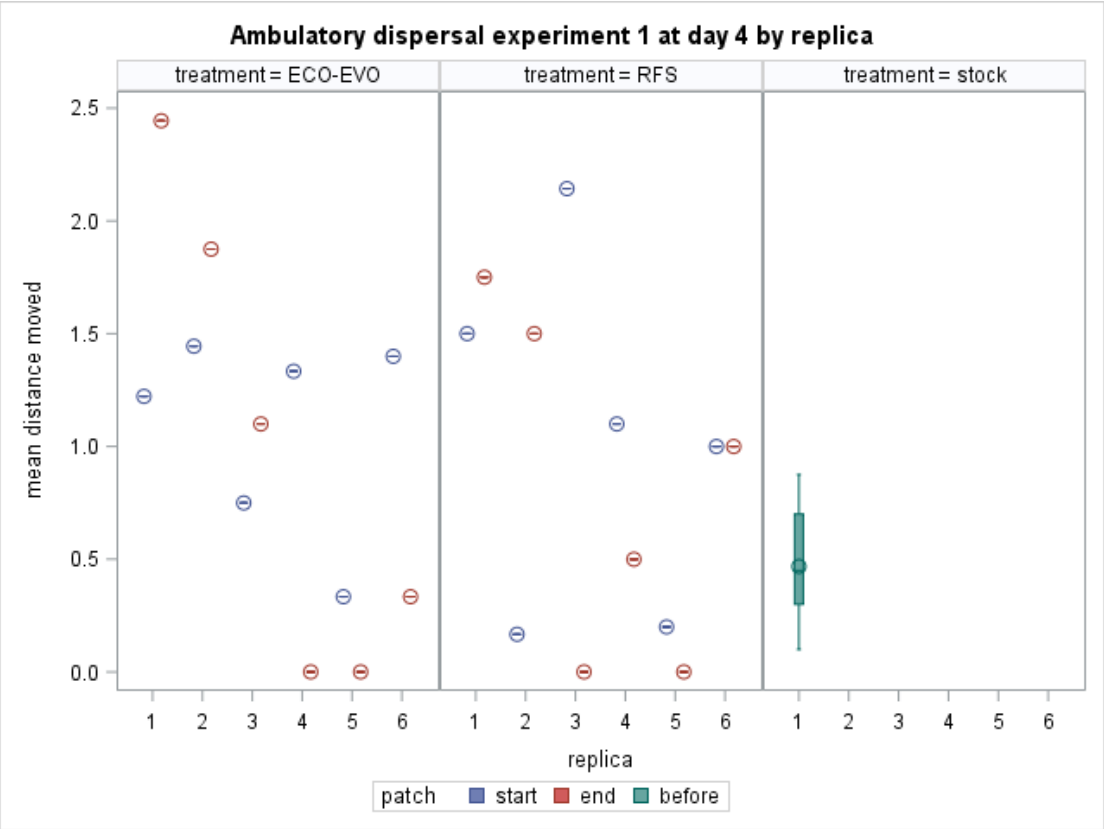
Experiment 2	ECO-EVO2	ECO
Start-end	$F_{1,17}$=2.58; p=0.1263	**$F_{1,17}$=4.91; p=0.0406**
End-stock	**$F_{1,20}$=5.11 p=0.0351**	$F_{1,4.03}$=2.22; p=0.2099
Start-stock	$F_{1,20}$=0.65; p=0.4296	$F_{1,3.93}$=0.74; p=0.4385

Appendix 1. 133: Statistical output for mean distance moved at the 4th day for experiment 2

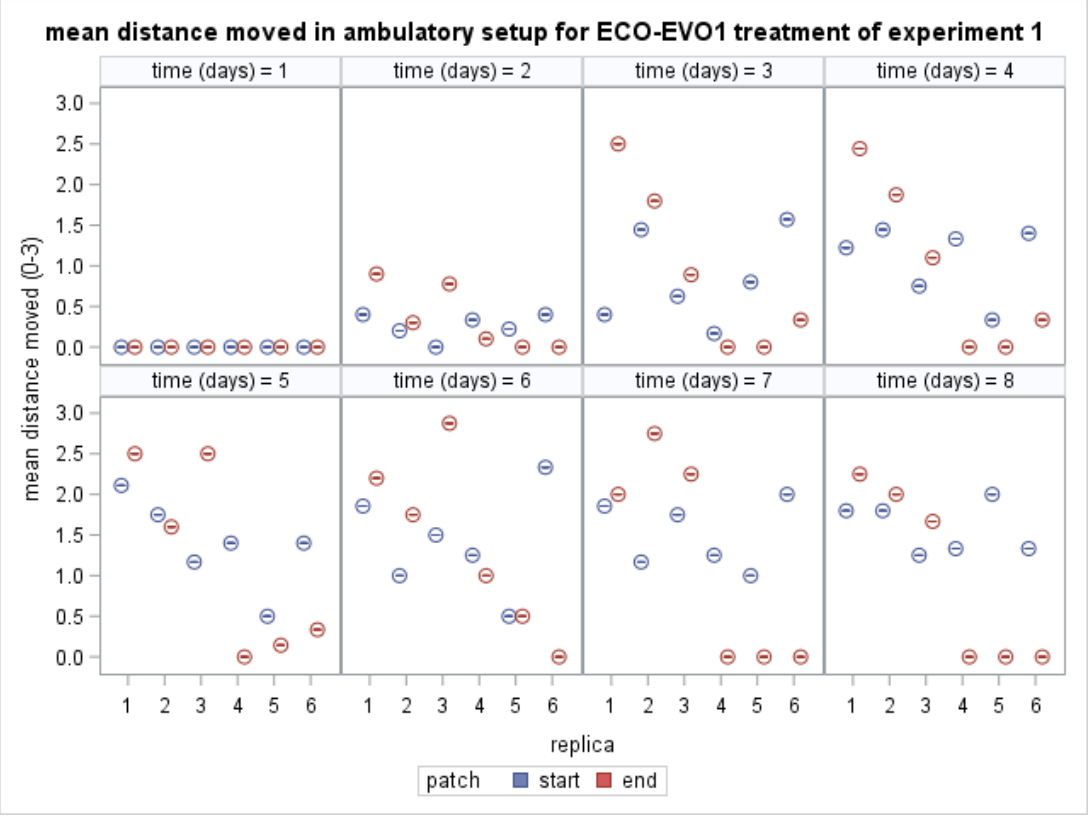
Mean distance moved at the fourth day differs significantly between the population from the core and the stock in the evolutionary unconstrained (ECO-EVO1) treatment of the first experiment, and between the population from the dispersal front and the stock in the evolutionary unconstrained treatment of the second experiment.



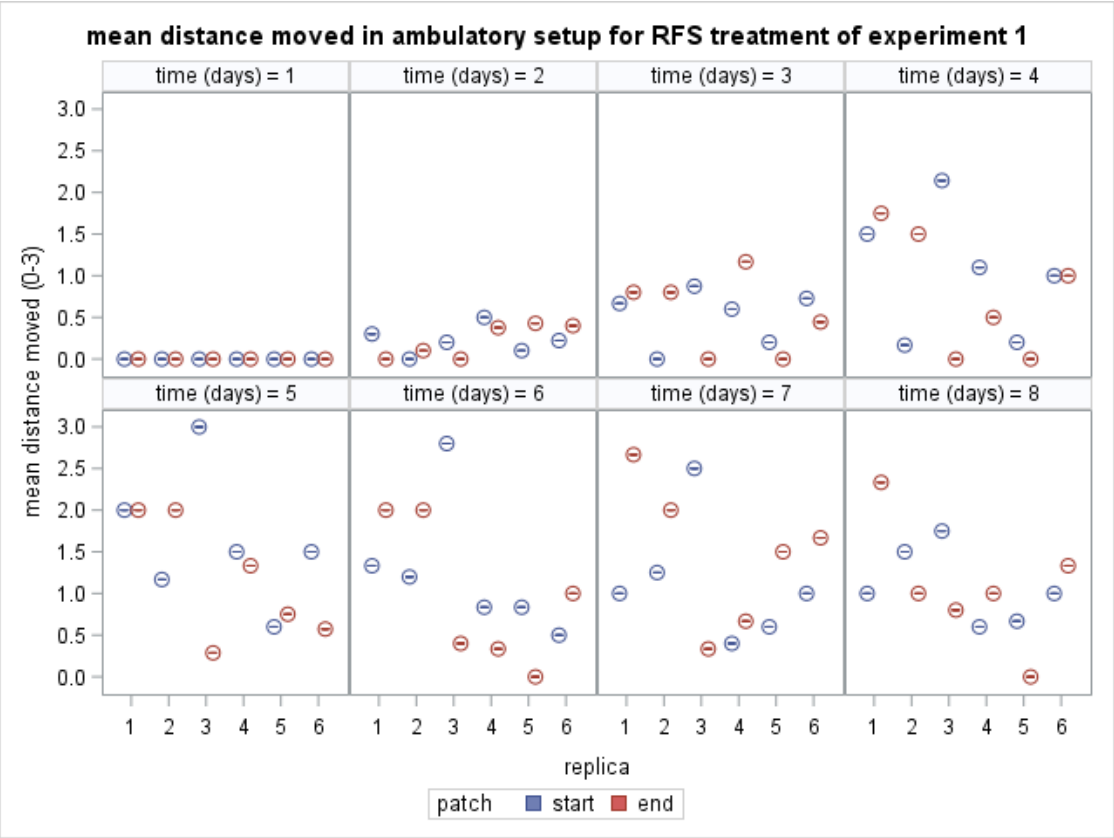
Appendix 1. 134: Mean distance moved on day 4 by treatment and patch for experiment 1



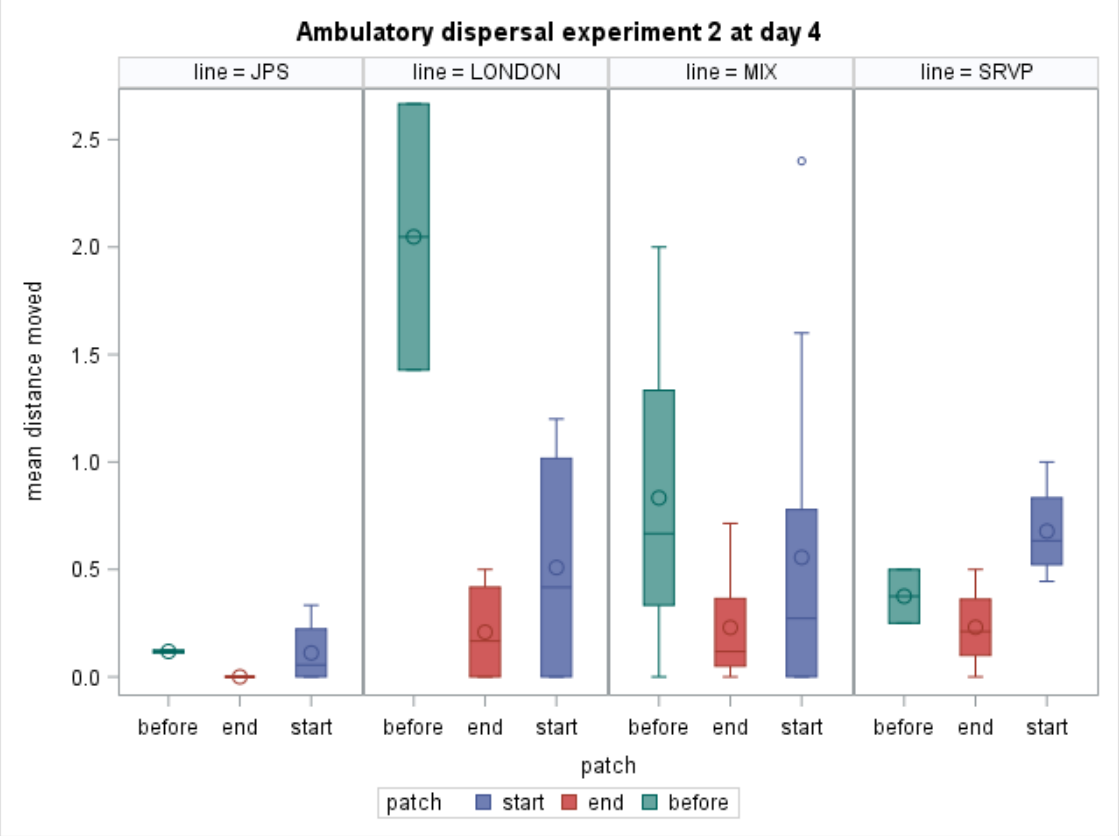
Appendix 1. 135: Mean distance moved on day 4 by replica and patch for experiment 1



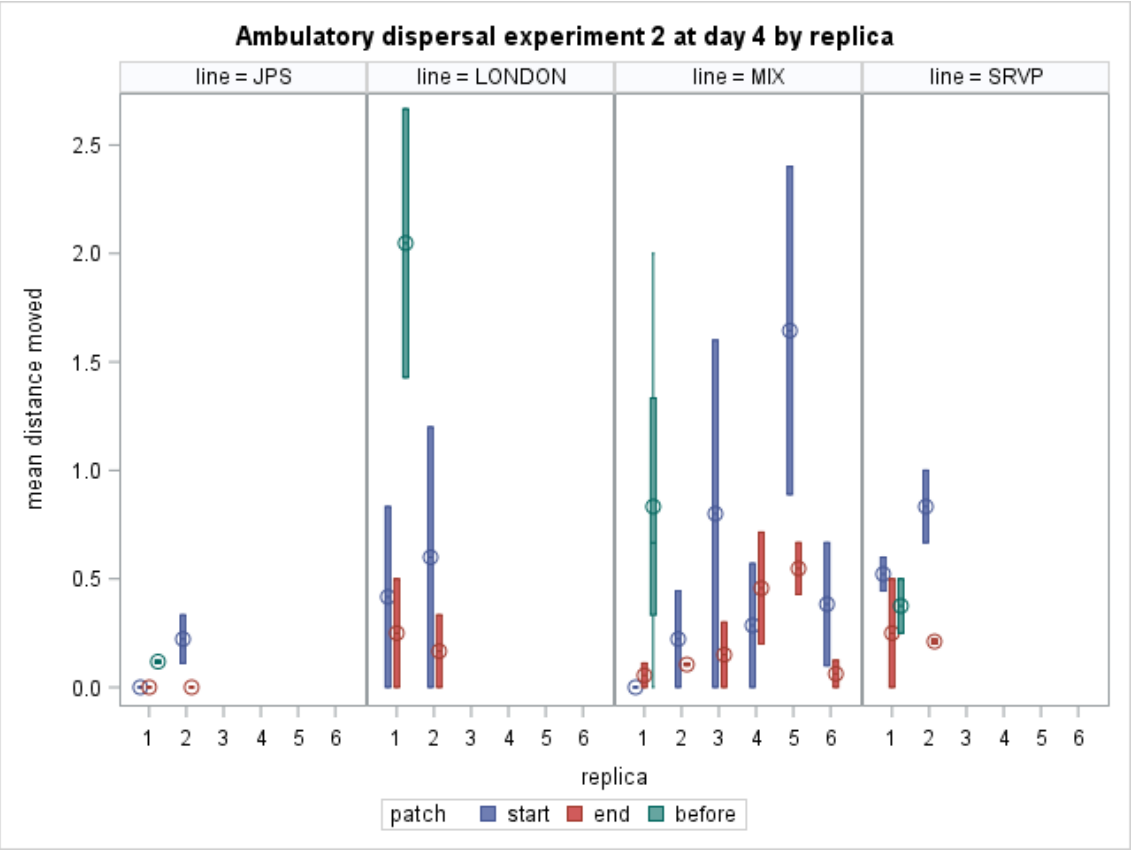
Appendix 1. 136: Mean distance moved at every day by replica and patch for the ECO-EVO1 treatment of experiment 1



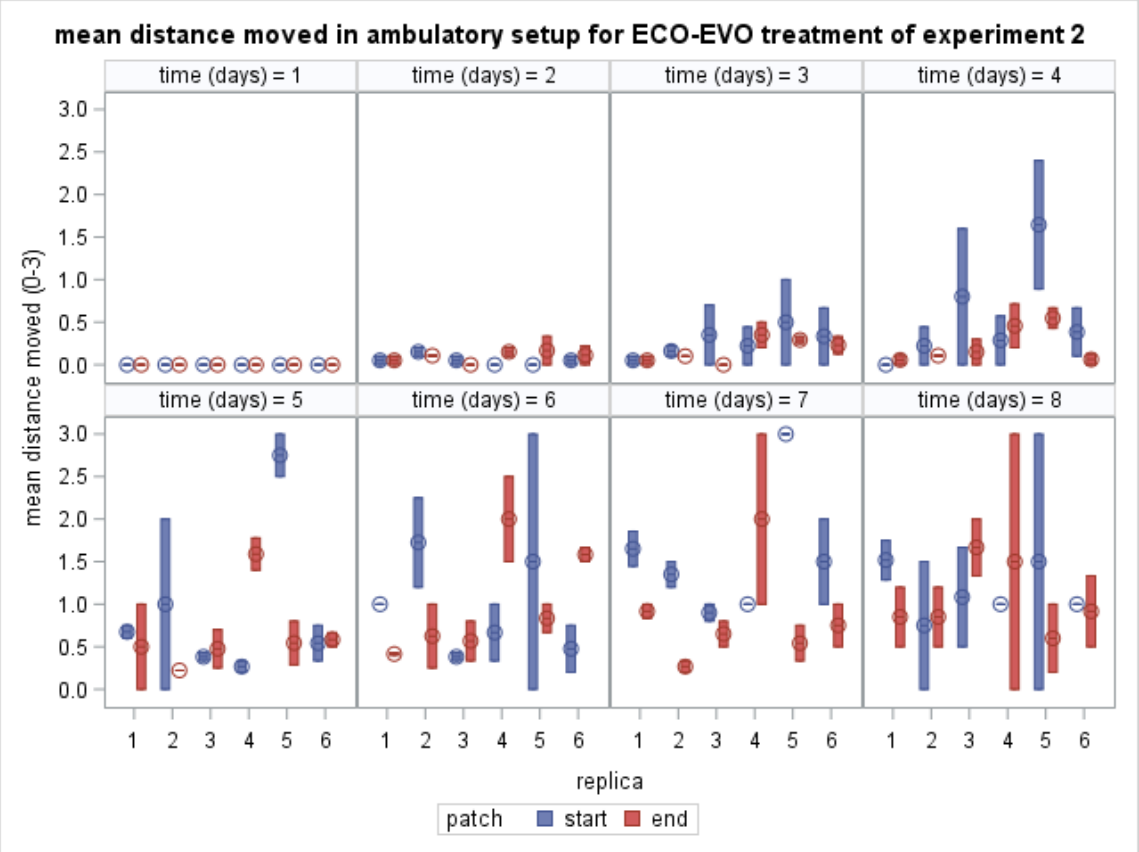
Appendix 1. 137: Mean distance moved at every day by replica and patch for the RFS treatment of experiment 1



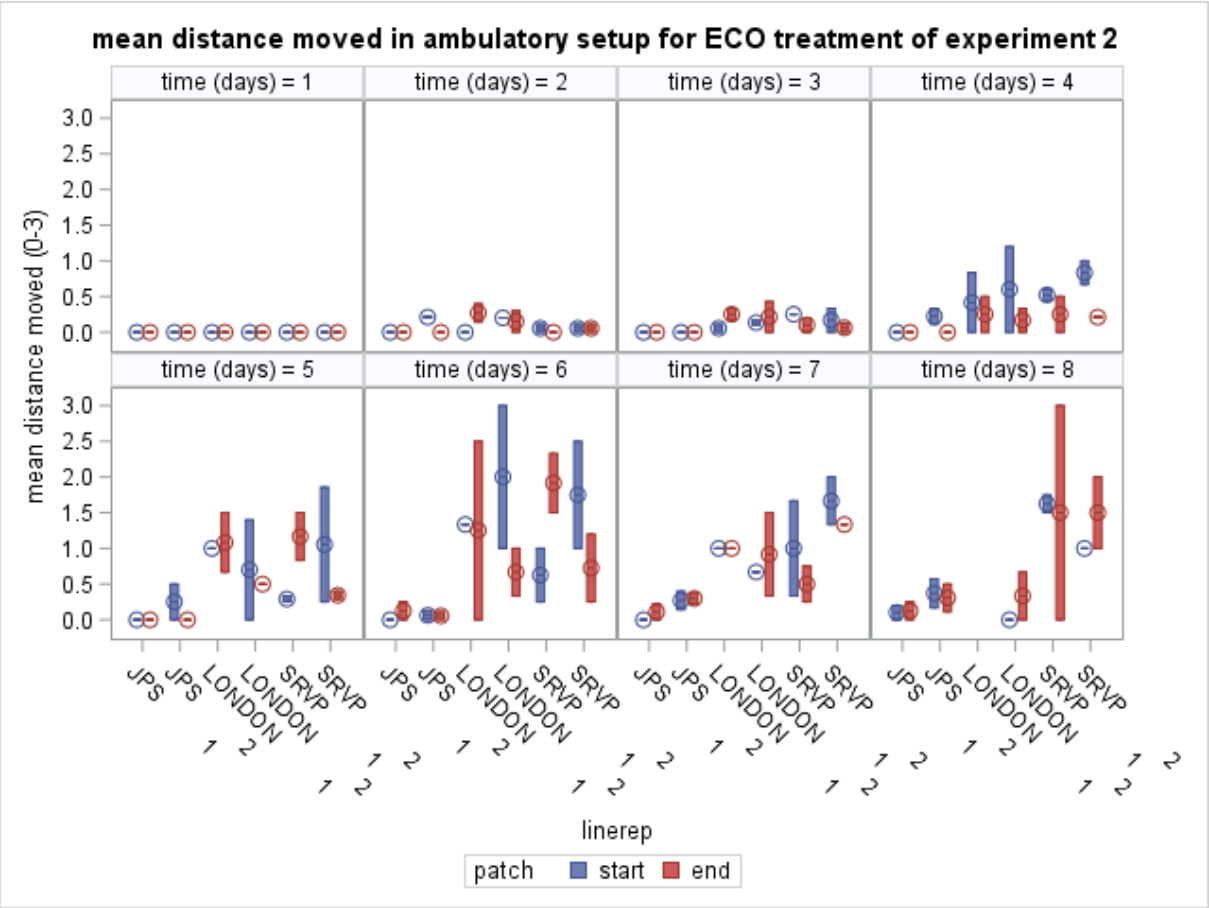
Appendix 1. 138: Mean distance moved on day 4 by line and patch for experiment 2



Appendix 1. 139: Mean distance moved on day 4 by replica and patch for experiment 2



Appendix 1. 140: Mean distance moved at every day by replica and patch for the ECO-EVO2 treatment of experiment 2



Appendix 1. 141: Mean distance moved at every day by replica and patch for the ECO treatment of experiment 2