International Institute for
Applied Systems Analysis
IIASA www.iiasa.ac.at

Post-zygotic reproductive isolation among populations of Iris atropurpurea: The effect of spatial distance among crosses and the role of inbreeding and outbreeding depression in determining niche width

Sapir, Y. and Mazzucco, R.

IIASA Interim Report
2012

Sapir, Y. and Mazzucco, R. (2012) Post-zygotic reproductive isolation among populations of Iris atropurpurea: The effect of spatial distance among crosses and the role of inbreeding and outbreeding depression in determining niche width. IIASA Interim Report. IIASA, Laxenburg, Austria, IR-12-064 Copyright © 2012 by the author(s). http://pure.iiasa.ac.at/10213/

Interim Reports on work of the International Institute for Applied Systems Analysis receive only limited review. Views or opinions expressed herein do not necessarily represent those of the Institute, its National Member Organizations, or other organizations supporting the work. All rights reserved. Permission to make digital or hard copies of all or part of this work for personal or classroom use is granted without fee provided that copies are not made or distributed for profit or commercial advantage. All copies must bear this notice and the full citation on the first page. For other purposes, to republish, to post on servers or to redistribute to lists, permission must be sought by contacting repository@iiasa.ac.at

International Institute for
Applied Systems Analysis
Schlossplatz 1
A-2361 Laxenburg, Austria

Tel: +43 2236 807 342
Fax: +43 2236 71313
E-mail: publications@iiasa.ac.at
Web: www.iiasa.ac.at

Interim Report **IR-12-064**

Post-zygotic reproductive isolation among populations of *Iris atropurpurea*: The effect of spatial distance among crosses and the role of inbreeding and outbreeding depression in determining niche width

Yuval Sapir
Rupert Mazzucco (mazzucco@iiasa.ac.at)

Approved by

Ulf Dieckmann
Director, Evolution and Ecology Program

February 2015

Interim Reports on work of the International Institute for Applied Systems Analysis receive only limited review. Views or opinions expressed herein do not necessarily represent those of the Institute, its National Member Organizations, or other organizations supporting the work.

Post-zygotic reproductive isolation among populations of *Iris atropurpurea*: the effect of spatial distance among crosses and the role of inbreeding and outbreeding depression in determining niche width

Yuval Sapir (1*) & Rupert Mazzucco (2)

(1) The Botanical Garden, Dept. of Molecular Biology and Ecology of Plants, Tel Aviv University, Ramat Aviv, Tel Aviv 69978 Israel. *Author for correspondence, e-mail: sapiry@post.tau.ac.il

(2) Evolution and Ecology Program, Institute for Applied Systems Analysis, Schlossplatz 1, 2361 Laxenburg, Austria

Keywords

Gene flow; Genetic spatial structure; Inbreeding depression; Oncocyclus irises; Outbreeding depression; Plant reproductive success; Reproductive isolation; Seed dispersal; Speciation;

Abstract

Question: What is the role of inbreeding and outbreeding depression in creating spatial patterns of reproductive isolation among populations within a species?

Hypothesis: A combination of inbreeding and outbreeding effects create an optimal crossing distance at which reproductive isolation is minimal.

Organism: *Iris atropurpurea* Dinsm., an endangered and endemic Israeli plant, with a fragmented distribution throughout the coastal plain.

Field sites: Two *I. atropurpurea* populations, one in the Shafdan dunes, and one in the Netanya Iris Reserve, both ca. 19 km S or N (respectively) of Tel Aviv, on the coastal plain in Israel.

Methods: We performed artificial cross-pollination within and between populations of *I. atropurpurea* at various distances and measured seed germination and seedling survivorship.

Results: Theoretical considerations led us to expect that inbreeding depression acts mostly at the small scale, and that higher offspring fitness is revealed at distances < 10 km. Results from the experiment showed that reproductive isolation acts differently in consequent stages of the hybrid life history. Pattern of total reproductive isolation among populations along a geographical axis showed different patterns in the two natural populations: while in the Netanya population no pattern appeared, in Shafdan we found a pattern of intermediate distance where reproductive isolation is the highest, and in short and long distances reproductive isolation relaxed.

Introduction

A key challenge for the study of speciation is to understand the development of reproductive isolating barriers (Coyne & Orr, 2004; Dobzhansky, 1940; Grant, 1981; Mayr, 1947). The separation of plant lineages by reproductive barriers is crucial for species maintenance, allowing independent evolutionary pathways to develop (Rieseberg *et al.*, 2006). Reproductive barriers among plant species can be broadly classified into three sequential stages. Firstly, pre-pollination isolating barriers can arise due to spatial distance (allopatry) or pollinator differentiation (Dell'Olivo *et al.*, 2011; Kay, 2006; Ramsey *et al.*, 2003; Stuurman *et al.*, 2004). Pre-pollination reproductive isolation can also be attained by morphological mechanisms that prevent heterospecific gene flow by differential deposition of the pollen and enhanced selfing by clustering of floral organs, even when visited by the same animal species (Scopece *et al.*, 2007; Smith & Rausher, 2007). Secondly, post-pollination pre-zygotic isolating barriers can arise, for example, due to effects on pollen competition (Dell'Olivo *et al.*, 2011; Lowry *et al.*, 2008a; Ramsey *et al.*, 2003). Thirdly, post-zygotic isolating barriers may act at the stage of the F1 hybrids or beyond. Post-zygotic barriers have been studied more than pre-zygotic ones (Lowry *et al.*, 2008a; Rieseberg *et al.*, 2006), but studies are now also accumulating on the relative importance of pre-zygotic barriers (Anderson *et al.*, 2010; Dell'Olivo *et al.*, 2011; Kay & Sargent, 2009; e.g., Lowry *et al.*, 2008b).

It has been argued that pre-zygotic isolating barriers are more important in speciation because they act earlier and block gene flow between species (Lowry *et al.*, 2008a). However, in the presence of gene-flow, post-zygotic isolating barriers are crucial for maintaining species boundaries (Smadja & Butlin, 2011). Contrary to earlier rejection of the idea of sympatric speciation (Mayr, 1942), it is now agreed that divergence among geographically close populations, or even within a population, is plausible (Pinho & Hey, 2010; Servedio & Noor, 2003; Smadja & Butlin, 2011). The focus of speciation studies may hence be directed to the level of populations (Ehrlich & Raven, 1969; Frankham, 2010) and to the role of ecological adaptation via natural selection in maintaining species boundaries (Morjan & Rieseberg, 2004; Rieseberg *et al.*, 2006). It is clear that speciation is a gradual process in time and in space, and that a continuous view should be taken in studying speciation driven by ecology (Hendry *et al.*, 2009; Hendry *et al.*, 2007; Nosil *et al.*, 2008; Nosil *et al.*, 2009; Rundle & Nosil, 2005; Schluter, 2009).

Reproductive isolation among populations within a species may depend on spatial niche heterogeneity. The pattern of reproductive isolation along a geographical distance axis in plant populations is a question of population divergence, which depends on the balance between gene flow and ecological adaptation (Doebeli & Dieckmann, 2003; Morjan & Rieseberg, 2004). Pollinator-mediated gene flow might be limited between distant populations, but advantageous alleles may spread among populations and maintain species homogeneity through natural selection (Morjan & Rieseberg, 2004). Connections between distant populations are made by stepping stone connections, enabling flow of advantageous alleles even between populations at the extremities of the distribution (Bossuyt, 2007). Even with gene flow between populations, not every cross has the same probability of success; inbreeding and outbreeding depression act to increase or decrease fitness in a cross between individuals from different populations (Devoto & Medan, 2004; Edmands, 2007; Waser & Price, 1989).

Inbreeding depression is the fitness reduction experienced by offspring resulting from a cross between genetically similar individuals (Byers & Waller, 1999; Charlesworth & Charlesworth, 1987). Inbreeding depression is usually explained as resulting from a heterozygote advantage over the homozygotes (over-dominance), or from deleterious mutations being expressed in the homozygote (partial dominance; Byers & Waller, 1999; Charlesworth & Charlesworth, 1987; Lynch *et al.*, 1995). With limited gene flow, crosses within a population are likely to be crosses between closely related genotypes. Hence, with inbreeding depression present, crosses within a population are expected to result in lower fruit-set and seed-set as compared to crosses between populations (Schleuning *et al.*, 2011; Segal *et al.*, 2007).

Outbreeding depression is the fitness reduction experienced by offspring resulting from a cross between genetically dissimilar individuals. It is proposed that the main reason for this fitness reduction is that dissimilar individuals are often in fact adapted to different environments, and that the hybrid offspring is then ecologically deficient in both parental environments (Lynch, 1991; Waser & Williams, 2001). Genetic distance increases with geographical distance (Jump & Peñuelas, 2006; Leblois *et al.*, 2006; Moyle, 2006), suggesting a correlation with local adaptation that will decrease fitness of hybrid crosses due to outbreeding depression. Indeed, a few studies have shown that maternal fitness decreases with increasing genetic distance to full hybrid sterility of the most distant populations, indicating an increase in outbreeding depression with crossing distance (e.g., Nosrati *et al.*, 2011; Pélabon *et al.*, 2005).

While under natural conditions hybrid fitness increase or decrease with distance may result from spatial speciation by means of reproductive isolation (Moyle, 2006; Stacy, 2001), in human-disturbed habitats this can be the result of fragmentation (Devoto & Medan, 2004; Jump & Peñuelas, 2006). A few studies have shown reduction of seed production coupled with low genetic variation in fragmented populations (Bossuyt, 2007; Fischer & Matthies, 1998; Schmidt & Jensen, 2000). Self-incompatibility coupled with genetic similarity among nearby populations can reduce fruit-set in fragmented populations, and require "genetic rescue" (Bossuyt, 2007). For example, the partial self-incompatible *Hypochoeris radicata* in Europe showed increased fitness when plants from different regions were crossed, compared to within-population crosses (Becker *et al.*, 2006). Similarly, in *Iris bismarckiana*, supplementary cross-pollination from a nearby population increased fruit- and seed-set, compared to within-population crosses (Segal *et al.*, 2007).

At present, niche theory predicts that crossing outside the niche will cause outbreeding depression, expressed as a deficiency of hybrids of crosses between plants outside the niche (e.g., Hoelzer *et al.*, 2008; Lenormand, 2012). This implies that selection acting on crosses among plants will increase with parental distance along the environmental gradient, because distance is potentially associated with environmental heterogeneity, hence a different ecological niche (Sambatti *et al.*, 2012; Volis, 2007). Despite the potential harm of outbreeding depression, if inbreeding is present, selection to avoid inbreeding might benefit crosses outside the niche and impede ecological speciation. Hence, it is important that inbreeding be integrated into niche theory.

Despite all of the above evidence for the role of distance between populations in reproductive success and reproductive isolation, there is no quantitative estimation of the relationship between them as a function of physical distance between mating individuals. In addition, the theoretical basis of such a relationship is lacking. Here we present results of both a spatial genetic model and an empirical study that examined this relationship. The goal of this study was to assess the relative importance of geographical distance on patterns of reproductive isolation among populations within a species. As a starting hypothesis we used the pattern proposed by Waser and Price (1989) and by Devoto and Medan (2004). They proposed an “optimal crossing distance”, and we consider cross success as conceptually reciprocal to reproductive isolation. Specifically we asked the following questions:

1) To what extent does post-zygotic reproductive isolation act between populations within a species?

2) Are patterns of reproductive isolation constant in space, or is there an optimal crossing distance?

3) Does reproductive isolation act differently on different fitness components?

4) How sensitive are the patterns of reproductive isolation to genetic and ecological parameters?

Methods

Study species

Iris atropurpurea Dinsm. belongs to the section *Oncocyclus*, which comprises ca. 30 species in the Middle East (Mathew, 1989). *I. atropurpurea* is endemic to the Israeli coastal plains, and its distribution is limited to a narrow belt of 70 x 12 km along the coast (Shmida & Pollak, 2007). The species grows solely on sandy soils of the Pleistocene age. This habitat is vulnerable due to its location in Israel's most populated area. The number of its populations is in decline, with about a third of the populations already extinct (Sapir *et al.*, 2003).

Oncocyclus irises, *I. atropurpurea* included, are self-incompatible and require insect pollination for fruit production (Sapir *et al.*, 2005). The nectarless flowers are pollinated by night-sheltering *Eucera* male bees, which enjoy the excess heat absorbed by the dark tepals following sunrise (Sapir *et al.*, 2006). Distance of pollen transportation is largely unknown, but anecdotal observations suggest that it usually no more than three hundred meters. The seeds are dispersed by ants and are carried up to 60 meters away from the plant (Y. Sapir, unpublished data).

Experimental design and crosses

In order to test the effect of crossing distance on reproductive success we performed a field experiment in February-March 2009. This experiment was performed in two populations of *I. atropurpurea*: Netanya (NET), which is located in the Northern part of the species range, and Shafdan (SHF), which is located in the Southern part (map in Figure 1). Developing buds were covered with insect-proof fine mesh until full wilting of the flower. Flowers were pollinated with pollen collected from other populations, covering the full range of the *I. atropurpurea* distribution, from Hadera to Ashdod. Anthers were collected during a few day trips from all populations in one region. The pollen was deposited on experimental flowers no later than 24 hours after pollen collection, by brushing the anthers from the donor flowers against all three large stigmas of the experimental flowers. Only one anther was used for pollinating each flower. The amount of pollen grains was sufficient to load the stigmas with >800 grains per stigma (checked with a field x20 microscope). A preliminary experiment showed that every 10 pollen grains deposited on the stigma are on average sufficient for producing one seed (Y. Sapir, unpublished data), hence, we expected to get >80 seeds from each hand-pollinated flower. Immediately after pollination, flowers were covered again to prevent uncontrolled natural pollination.

The distances of the pollen-donor populations to both experimental sites were estimated to the nearest 0.1 km. Pollen-donor populations were classified into two categories, “near” or “far”, relative to the experimental sites, based on their location North or South of Tel Aviv, which is located in the approximate center of the distribution of *Iris atropurpurea*. Donor populations were categorized as “near” if they were in the same region as the acceptor population, or “far” if they were in the other side of Tel Aviv.

Six weeks after pollination, the capsules were collected from the experimental flowers and dried at room temperature until full maturation of the seeds. Fruit-set was calculated as the percentage of flowers that had produced capsules. All seeds were counted. Flowers that produced capsules with no mature seeds were considered as a fruit with zero seeds, indicating successful pollination, but failure of fertilization.

Seeds were pooled by the origin of their pollen donor for each maternal population (NET or SHF). Two hundred and fifty seeds from each cross treatment (i.e., the same maternal and the same donor populations) were sown in October 2009 into 10 x 10 x 15 cm pots, fifty seeds in each pot. Pots were located in a net house at the Tel Aviv University Botanical Garden, and watered with mist sprinklers for 12 minutes, three times a week. Germination was recorded weekly until 30 April 2010. When the first leaf appeared (usually a week after emergence), the seedling was transferred to another pot of the same size, with commercial nutrient-rich soil. Five or six seedlings of the same cross were planted together in each pot, to mimic (mild) sibling competition. Because first year germination was very low (~2%; data not shown), the seeds were allowed to germinate in the next season (October 2010 to April 2011). The total germination in both years was 14%.

Seedlings that germinated in the 2009/2010 season were planted in November 2010 into a semi-natural habitat within the Tel Aviv University Botanical Garden. The habitat is a remnant of the original sand-stone ecosystem, typical of Israel's coastal strip, with a shallow sandy soil layer over the sandstone bedrock. This habitat is relatively stressful, due to rapid drainage of rain water through the sand and stone. The vegetation consists of a few *Coridothymus capitatus* dwarf shrubs and herbaceous plants, dominated by the invasive *Oxalis pes-caprae*. Seedlings were planted in a fully randomized array, with 20 cm distance between plants. In November 2011 survivorship of plants was recorded.

Data analysis

All statistical analyses were done using R (R Development Core Team, 2011). Cross treatment was defined as a single combination of maternal population (NET or SHF) and a pollen-donor population. Fruit-set was calculated as the fraction of flowers that set fruits within each cross treatment. Seed-set was calculated as the average number of seeds per capsule for each cross treatment. Fraction of germination was calculated as the average percentage of seeds germinated in five pots from each cross treatment, pooled over the two years of germination. Seedling survival was calculated as the average fraction of seedlings surviving from each cross treatment one year after planting.

Pollen-donor populations were categorized as “near” and “far”, relative to their distance from the experimental site. Differences among cross treatments in each of the above four parameters were tested by nested analysis of variance (ANOVA), with donor population nested within region (near or far), which, in turn, nested within experimental site (NET or SHF). Significance was tested assuming a binomial distribution for fruit-set and a Poisson distribution for seed-set. Differences between crosses within the population and from near or far populations were tested by ANOVA with post-hoc contrast analyses in cases where ANOVA showed a significant regional effect.

The absolute reproductive isolation (RI) in each of the sequential life stages, as well as total RI, were calculated following Ramsey et al. (2003). In short, the

RI for each life stage is 1-($X_{\text{crosses between populations}}$ / $X_{\text{crosses within populations}}$). The absolute RI is defined as the absolute contribution (AC) of each stage, after accounting for the effect of the previous stage: AC_1=RI_1; AC_2=RI_2(1-AC_1); AC_3=RI_3[1-(AC_1+ AC_2)], etc. Total RI was calculated as the sum of the four absolute RIs. Although RI values are expected to range from 0 to 1, we allowed these values to be negative, because in our approach negative values are informative as well, representing greater success of crosses between populations, relative to crosses within populations.

Theoretical considerations

Now we turn to the question of whether we can estimate the fitness effect as a function of crossing distance and the location of the expected fitness peak, that is, the optimal crossing distance. The basic problem here is that ideas about inbreeding and outbreeding effects (Devoto & Medan, 2004; Waser & Price, 1989) are rather generic and qualitative, and little else is known; to extract some quantitative insight, we will have to rely on the following assumptions:

The first assumption concerns the environmental structure that affects hybrid fitness via maladaptation to the local (maternal) environment, leading to outbreeding depression. It is not obvious which phenotypic trait of the plant, or which combination of traits, should confer this adaptive quality, but we shall stick with the common assumption that a quantitative trait exists to this effect (Sun & Cheptou, 2012), and that the locally optimal trait value varies linearly in space, in our case, along the North-South gradient along the Israeli coast (Figure 1). The environmental structure is thus described by specifying the slope of this linear environmental gradient, that is, the change in phenotypic trait value per change in spatial distance.

The second assumption concerns the effect of maladaptation on fitness. By definition, locally non-optimal phenotypes experience reduced fitness. We make the common assumption that the decrease in relative fitness of a non-optimal type with deviation from the locally optimal type is described by a Gaussian function, the width of which is constant along the gradient. Therefore, we can use this width to scale all trait values, and restrict our further discussion to dimensionless scaled trait values, which also releases us from the need to speak about units and reasonable magnitudes of this unknown trait.

A relevant spatial scale is provided by the average pollination distance, which we can likewise use to introduce dimensionless scaled spatial distances, such that a distance $x < 1$ implies that cross-pollination is still rather frequent under natural conditions; this also defines what “local” means in practice. We neglect seed transport, which occurs over much smaller distances (by a factor of ~ 10).

Fitness effect of the environmental gradient is “not obvious”; our experimental procedure relied on this when seedlings were transplanted from the experimental sites to the botanical garden in Tel Aviv, where we expected their growth and survival to be a reasonable proxy for their fate at the experimental site. This translates into the sub-assumption that the slope of the environmental gradient cannot be too steep, but must be rather shallow, that is, $s \ll 1$. It is well known (e.g., Kirkpatrick & Barton, 1997) that on such shallow gradients, the adaptive equilibrium is a phenotypic cline that follows the gradient, that is, with lack of gene flow the dominant phenotype at each location is the locally most adapted. Indeed, at the large scale such a phenotypic cline is apparent in *Oncocyclus* irises along a climate gradient (Arafeh *et al.*, 2002; Sapir *et al.*, 2002).

With all these assumptions, we can directly estimate the outbreeding depression effect as the relative local fitness of a phenotypically intermediate hybrid resulting from pollination from a distance x as $w_{\mathrm{E}}(x) \approx \exp(-x^2 s^2 / 8)$.

The hybrid fitness peak we are looking for is, as a generic approximation, the maximum of the function $w(x) = w_{\mathrm{E}}(x) w_{\mathrm{G}}(x)$, in which the environmental hybrid fitness (i.e., extrinsic reproductive isolation based on ecologically-dependant fitness) is multiplied with the "genomic fitness" w_{G} of a hybrid, which describes the effect of inbreeding depression and is an intrinsic, ecologically-independant reproductive isolation. We know little about this function, other than that it should assign a reduced relative genomic fitness $h < 1$ to local crossings, and that it should saturate at 1 as genetic differences between flower and pollen donor accumulate with distance, a behavior that is, for example, exhibited by the Holling-Type-II-like function $w_{\mathrm{G}}(x) = h + (1-h)/(1+g/x)$. The distance g here is the crossing distance at which half of the depression effect is overcome.

This maximum of w lies in the region where both w_{E} and w_{G} are sufficiently high, and as shown in Figure 2a, it could potentially be very pronounced. But here we encounter the first problem: the shallow slope of the environmental gradient implies that the bulky region of w_{E} extends over a large distance, so that the outbreeding depression effect does not constrain the location of the fitness peak very much (Figure 2b,c), or define the optimal crossing distance very well (if the invariably present stochasticity is also taken into account).

The shape of the genomic fitness function w_{G}, describing inbreeding depression, is determined by the two parameters h and g. We have no *a priori* information about either of them, but we can guess that h is probably not too small, that is, closer to 1 than to 0, because after all, local crosses are not doing too badly overall. This is not surprising, because these values should be under strong selection – if local crosses are common, it is clearly not advantageous if they have to pay a high fitness penalty. For the same reason, we should expect g to have evolutionarily attained the smallest possible values (these considerations are formalized by calculating the expected genomic offspring fitness as a function of h and g, Figure 2, inset). If there now exist genetic constraints that somehow prevent this smallest possible value from being equal to 0 (lifting any penalty on purely local crossings and making inbreeding depression vanish), it stands to reason that it should be a small multiple of the pollination distance, i.e., $g \geq 1$, where some genetic differences can already accumulate and may provide enough variation to counter the deleterious effects of local crossings.

In summary, our generic expectation from the above considerations is that the fitness effect with distance should be weak (Figure 2c). It reveals an increase in hybrid fitness with increasing crossing distance toward the fitness peak, which we would expect to occur on a relatively short spatial scale relative to the average pollination distance. Note that the effect demonstrated in this model is of the hybrid fitness, which may correspond reciprocally to reproductive isolation among populations, as tested empirically here.

Results

Field experiment - Fruit-set

Fruit-set was 0.84 in Netanya (NET), and 0.92 in Shafdan (SHF). Analysis of variance revealed a significant effect of experimental site on fruit-set, but not of the region or donor population (Table 1).

Analyzed separately for each site, fruit-set did not differ between crosses with near and far populations, or between both crosses and fruit-set in crosses within populations for both sites (ANOVA with binomial distribution – NET: $F_{2,149}=1.40$, $P=0.25$; SHF: $F_{2,206}=2.92$, $P=0.056$; Figure 3). Fruit-set among pollen-donor populations at different distances was not significantly different in NET (Table 2), and was marginally significantly different in SHF (Table 3), mainly due to low fruit-set in flowers pollinated with pollen from Kadima (Contrast analysis: $P<0.05$).

Seed-set

Analysis of variance revealed a significant effect of the region of donor population (nested within experimental site) on seed-set, but not of the specific donor population (Table 1). Analyzed separately for each site, seed-set was higher in NET in crosses with near populations, relative to crosses within the population or with far populations, but this difference was not significant (ANOVA with Poisson distribution: $P=0.131$; Figure 3). In SHF, seed-set for crosses within the population was significantly lower than for crosses between populations, both far and near (Figure 3). Seed-set among pollen-donor populations at different distances was not significantly different in NET (Table 2), and only marginally significantly different in SHF (Figure 3). As with fruit-set, this difference was mainly due to extreme low seed-set in flowers pollinated with pollen from Kadima (Contrast analysis: $P<0.05$). In these crosses with Kadima, most fruits that were produced did not set any seed.

Germination

Analysis of variance revealed a significant effect of the region and of the specific donor population (nested within experimental site) on fraction of seed germination (Table 1).

Analyzed separately for each site, germination in NET was different between seeds from crosses with far and near populations, and germination of seeds from crosses within the population was similar to both categories of crosses between populations (Contrast analysis: $P<0.05$; Figure 3). In SHF, germination was significantly higher for crosses within the population, relative to germination of seeds from crosses between populations (Contrast analysis: $P<0.05$; Figure 3). Fraction of seed germination was significantly different among pollen-donor populations at different distances in both sites (Table 2, Table 3).

Survivorship

Fraction of survived seedlings from SHF was higher than from NET (0.69 and 0.61, respectively). Analysis of variance revealed a significant effect of the experimental site and of the specific donor population (nested within experimental site) on fraction of seedling survival (Table 1). Analyzed separately for each site, seedlings from both sites did not differ significantly in survivorship between crosses from near or far populations, or crosses within populations (Figure 3). Seedling survivorship was different among crosses in NET (Table 2), but without a clear spatial pattern. For seedlings from SHF, the effect of pollen-donor population was marginally significant, and without a clear spatial pattern, as in NET (Table 3).

Reproductive isolation

Absolute reproductive isolation (RI) for all four fitness components was not significantly associated with distance of pollen-donor population. However, although not significant, general trends were slightly different among components and sites (Figure 4). Note that here we did not use regression functions to estimate effect size due to the very small number of points for each component; instead, we looked for a general trend in the relationship between RI and distance of the crossed plants. When the single outlier point (Kadima population) was removed, RI based on fruit-set did not change with distance in SHF, while in NET there was an increase of RI with distance (Figure 4, top left). Slopes of RI based on seed-set as a function of distance were positive for both NET and SHF, and the difference between the two slopes was marginally significant (P=0.084; Figure 4, top right). RI based on fraction of germination had a similar negative linear trend in both sites (Figure 4, bottom left). However, while the slope was not significantly different from zero in NET (P=0.479), it was significantly different from zero in SHF (P=0.023). Absolute RI based on seedling survival did not show any trend with distance between populations, and was not different from zero (Figure 4, bottom right).

Absolute RI was similar in value and direction in crosses with near and far populations in SHF (Figure 5, top). However, in NET we found a mirror image of RI values for crosses with near and far populations for all four RI components (Figure 5, bottom).

Total RI values were not associated with distance of pollen-donor populations for crosses in NET (Regression analysis: P=0.49; Figure 6). In SHF, total RI had no significant linear association with distance, but a negative quadratic term was marginally significant (P=0.053), with a peak at a distance of 29.3 km (Figure 6).

In NET, Total RI was negative (-0.28) for crosses with near populations, and close to zero (0.01) for crosses with far populations. In SHF, total RI was very close to zero (-0.0004) for crosses with near populations, while it was positive (0.25) for crosses with far populations.

Discussion

Spatial pattern of reproductive isolation

Reproductive isolation is an important mechanism of speciation, and considered to be associated with both inbreeding and outbreeding depression (Forrest *et al.*, 2011; Lynch, 1991; Stacy, 2001; Waser & Price, 1989). The reciprocal effect, reproductive success, was proposed to have an optimum at an intermediate distance due to the combined effect of inbreeding and outbreeding depression (Devoto & Medan, 2004; Paschke *et al.*, 2002; Waser & Price, 1989). Here we provide evidence that reproductive isolation within a species is plausible, but reproductive success does not have an optimal cross distance at a scale of tens of kilometers. Instead, our results suggest that the existence and location of this optimum may be at a very local scale, and depends on parameters such as local adaptation and dispersal. Our theoretical considerations suggest that under natural pollination conditions, hybrid fitness (which roughly correspond to the reciprocal of post-zygotic reproductive isolation) is lowest at short distances, while at intermediate and long distances it depends on the steepness of the ecological gradient and on the extent of inbreeding depression and genetic distances among plants (Figure 2).

Our initial hypothesis was based on results from studies that found an optimal crossing distance (Devoto & Medan, 2004; Forrest *et al.*, 2011; Paschke *et al.*, 2002; Waser & Price, 1989). In all these studies, however, the distances of the crosses were relatively short and categorical, while our study is at least one order of magnitude greater and on a continuous scale. For example, Forrest et al., (2011) tested the effect of distance on mating success at categorical distances ranging from 1 meter to 5 km. Waser and Price (1989) crossed plants from distances of 1, 10, or 100 meters. None of the above cited studies reached tens of kilometers or used a continuous scale, as our study did. Both the model and the empirical study suggest that there might be a short-distance pattern that increases hybrid fitness with distance, towards a local optimal crossing distance, but at the larger scale there is a constant value for a relatively long distance (Figure 2). We argue that our model provides a better hypothesis for the spatial pattern of mating success and post-zygotic isolation.

In *Iris atropurpurea*, we found that one population had no spatial pattern of reproductive isolation as a function of distance, while the other population exhibited an intermediate distance at which reproductive isolation reached a maximum (Figure 6). This maximum is relatively high and may create a barrier to gene-flow within the species. In line with our theoretical considerations, we suggest that this maximum RI at an intermediate distance is the result of maximum genetic distance among populations at an intermediate geographical distance, perhaps due to heterogeneity of the habitat.

Different RI with different fitness components

The extent and pattern of RI within a spatial scale were different when different fitness components were used. For example, absolute RI increased with distance between populations when number of seeds was considered (Figure 4, top right). A decrease in the number of seeds is affected by inbreeding depression, resulting from genetic similarity between plants (Charlesworth & Charlesworth, 1987; Shi *et al.*, 2005). Conversely, a decrease in RI with distance was found for seed germination (Figure 4, bottom left), another signal for inbreeding depression. The latter trend complies with the hypothesis that inbreeding depression decreases with distance among plants (Devoto & Medan, 2004; Stacy, 2001; Waser & Price, 1989). Hybrid viability is a signal for the effect of outbreeding depression and offspring survivorship is expected to decrease with distance among plants (Devoto & Medan, 2004; Forrest *et al.*, 2011; Lynch, 1991; Pélabon *et al.*, 2005). In *Iris atropurpurea*, the two maternal populations showed an interesting difference in pattern of offspring survivorship as a function of distance among the parents (Figure 4). While in SHF there was no effect of distance on the extent of RI, an increase of RI with distance was found in NET (Figure 4, bottom right).

The difference in the spatial pattern of RI between the two maternal populations was more pronounced when pollen-donor populations were clustered by region. While in SHF the extent of RI did not differ between near and far populations (Figure 5, bottom), in NET the direction of RI was opposite for crosses with near and far populations in three out of four fitness components (Figure 5, top). For example, germination was higher in crosses with populations that are far from NET, compared to within-population crosses (=negative RI), while in crosses with near populations germination was lower than within-population crosses (=positive RI). This may provide evidence that inbreeding depression in *I. atropurpurea* acts at the germination stage, while outbreeding depression acts at other life history stages of the hybrid. Our results of hybrid vigor in crosses with far populations, expressed by seed germination, are in agreement with the pattern found in two other studies, on tropical trees (Stacy, 2001) and on a European herb (Becker *et al.*, 2006).

Seed production and seed germination serve as strong post-zygotic reproductive isolation barriers when pre-zygotic isolation barriers are lacking (Costa *et al.*, 2007). Empirical studies showed that the relative contribution of pre-zygotic isolation barriers is more important in maintaining species boundaries (Lowry *et al.*, 2008a). These estimations, however, do not account for processes within a species, and usually ignore population differentiation (Ehrlich & Raven, 1969). Our results suggest that within-species processes of gene flow and the build-up of reproductive barriers can be important for either ecological speciation, or species homogenization, depending on the scale of gene flow.

Reproductive isolation and conservation

Conservation managers are faced with a very difficult dilemma: to promote among-population hybridization for genetic rescue from inbreeding depression, or to avoid such hybridization for fear of inducing outbreeding depression (Edmands, 2007; Frankham, 2010). While much evidence exists for the former, the latter is still lacking strong empirical support (Edmands, 2007). Although our study tests inbreeding and outbreeding depression, both empirically and theoretically, it does not provide a general decision criterion. We found differences in the patterns of reproductive isolation among the two tested populations of *Iris atropurpurea*, which imply different conservation management programs. While in SHF there was an advantage for crosses with nearby populations relative to crosses within populations, in NET there was no such fitness advantage. Instead, crosses with far populations were disadvantageous relative to crosses within the population or crosses with near populations. These results suggest that for increasing overall success of SHF population there is a need to import pollen from nearby populations, while in NET crosses within the populations are sufficient. These results also point to a metapopulation genetic structure. Bossuyt (2007) found that pollination outside a metapopulation increased fitness. In that study, lack of compatible pollen was responsible for lower reproductive success, which suggests that reproductive isolation within metapopulations is due to inbreeding depression. Our results show the opposite – total RI was higher for crosses with far populations, suggesting that outbreeding depression is the major force acting on *Iris atropurpurea* populations and that pollination within a metapopulation is the preferred conservation practice. There are only a few studies in plants that showed a disadvantage of crosses within the population compared to crosses among populations (e.g., Becker *et al.*, 2006; Kolb, 2005), and here we provide another example. However, the predominance of studies that show increase of hybrid fitness in crosses between populations (or metapopulations) suggests that inbreeding depression is dominant over outbreeding depression in shaping the spatial pattern of reproductive isolation among plant populations, with the irises as an exception.

The results of this study also have application for dealing with the risk of habitat fragmentation, which is considered to be a great threat on ecosystem functioning and species persistence (Saunders *et al.*, 1991; Wilcox & Murphy, 1985). Fragmentation affects population size and is predicted to decrease genetic diversity within populations (DiBattista, 2008) due to reduced effective population size, resulting in increased inbreeding depression (Ellstrand & Elam, 1993; but see Leimu *et al.*, 2006). Fragmentation can also increase among-population genetic distance due to reduced gene flow coupled with local adaptation (DiBattista, 2008; Willi *et al.*, 2007), resulting in outbreeding depression in the rare cases of crosses between populations. Conservation management programs aim to prevent these effects of fragmentation by means of “genetic rescue” of artificial gene flow between populations (Bossuyt, 2007; Frankham, 2010; Segal *et al.*, 2007). However, this genetic rescue may be unnecessary in some cases (Leimu *et al.*, 2006). For example, genetic diversity of *Lupinus oreganus* does not indicate a fragmentation-driven genetic bottleneck (Severns *et al.*, 2011). Similarly, our results suggest that fragmentation of *Iris atropurpurea* in NET population has not resulted in a reduction in genetic diversity to the level that inbreeding depression is acting on crosses within the population. In the other population (SHF), however, there is an advantage for crosses from nearby populations, similar to the finding in *Iris bismarckiana* (Segal *et al.*, 2007), implying that fragmentation is a threat to population dynamics.

Optimal crossing distance between populations, which is the distance of maximum probability of reproductive success (Devoto & Medan, 2004; Forrest *et al.*, 2011; Paschke *et al.*, 2002; Sobrevila, 1988; Waser & Price, 1989), may serve as an empirical proxy for the minimum distance without fragmentation required to maintain a population’s viability (Van Geert *et al.*, 2010). According to this line of thinking, fragmentation threatens plant population dynamics if it disconnects populations located at a distance shorter than the optimal crossing distance (Kolb, 2005; Volis *et al.*, 2005). We can draw such conclusions from our theoretical (model) results, which suggest that the highest fitness of crosses between *Iris* plants is at the shortest distance. Indeed, this supports the fundamental policy of connectivity between populations as a tool for conservation (Tabarelli & Gascon, 2005). The empirical data for SHF also support the need to prevent fragmentation at the small scale. However, the results from NET do not show a potential risk of fragmentation, because reproductive isolation is similar (or non-exist) at all spatial scales.

To summarize, we found an empirical pattern of reproductive isolation within a species in *Iris atropurpurea*, but consistency among maternal populations of this species was lacking. This could be due to differences in the parameters that shape the offspring fitness function in space, namely inbreeding depression, outbreeding depression and the pollination kernel, as demonstrated in the spatial genetic model. We conclude that small-scale patterns of reproductive isolation are sensitive to the genotypic architecture of fitness-related traits and the spatial genetic structure of the populations. This also has implications for conservation and preservation practices for endangered plant species, such as *Iris atropurpurea*.

Acknowledgements

We thank Géza Meszéna and organizers of the Niche Theory and Speciation Workshop for the opportunity to present this research and for inspiring discussions. A. Alagem, O. Har-Edom and I. Gerlitz assisted in field work. R. Cohen and O. Ziv helped with the germination and seedling survivorship experiments. Special thanks to B. Segal who provided great ideas for the conceptual framework of this study. We thank N. Tessler for producing the map in Fig. 1. G. Meszéna and A. Hendry provided useful comments on the manuscript, and C. Golodets helped with English editing. Logistic and financial support was provided by the Tel Aviv University Botanical Garden. This research was supported by a grant from Israel Science Foundation and by Porter School for Environmental Studies at Tel Aviv University. This is publication 12-01 from Tel Aviv University Botanical Garden.

Bibliography

Anderson, B., Alexandersson, R. and Johnson, S.D. 2010. Evolution and coexistence of pollination ecotypes in an African *Gladiolus* (Iridaceae). *Evolution,* **64**: 960-972.

Arafeh, R.M.H., Sapir, Y., Shmida, A., Iraki, N., Fragman, O. and Comes, H.P. 2002. Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. *Oncocyclus* = the Royal Irises) along an environmental gradient in Israel and the West Bank. *Molecular Ecology,* **11**: 39-53.

Becker, U., Reinhold, T. and Matthies, D. 2006. Effects of pollination distance on reproduction and offspring performance in *Hypochoeris radicata*: Experiments with plants from three European regions. *Biological Conservation,* **132**: 109-118.

Bossuyt, B. 2007. Genetic Rescue in an Isolated Metapopulation of a Naturally Fragmented Plant Species, Parnassia palustris. *Conservation Biology,* **21**: 832-841.

Byers, D.L. and Waller, D.M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics,* **30**: 479-513.

Charlesworth, D. and Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics,* **18**: 237-268.

Costa, C.B.N., Lambert, S.M., Borba, E.L. and de Queiroz, L.P. 2007. Post-zygotic Reproductive Isolation Between Sympatric Taxa in the *Chamaecrista desvauxii* Complex (Leguminosae-Caesalpinioideae). *Ann Bot,* **99**: 625-635.

Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Dell'Olivo, A., Hoballah, M.E., Gübitz, T. and Kuhlemeier, C. 2011. Isolation barriers between *Petunia axillaris* and *Petunia integrifolia* (solanaceae). *Evolution,* **65**: 1979-1991.

Devoto, M. and Medan, D. 2004. Preliminary evidence of landscape-level structure in a population of a perennial herb, *Cypella herbertii* (Iridaceae). *Ecologia Aplicada,* **3**: 188-191.

DiBattista, J. 2008. Patterns of genetic variation in anthropogenically impacted populations. *Conservation Genetics,* **9**: 141-156.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. *The American Naturalist,* **74**: 312-321.

Doebeli, M. and Dieckmann, U. 2003. Speciation along environmental gradients. *Nature,* **421**: 259-264.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology,* **16**: 463-475.

Ehrlich, P.R. and Raven, P.H. 1969. Differentiation of populations. *Science,* **165**: 1228-1232.

Ellstrand, N.C. and Elam, D.R. 1993. Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annual Review of Ecology and Systematics,* **24**: 217-242.

Fischer, M. and Matthies, D. 1998. RAPD variation in relation to population size and plant fitness in the rare Gentianella germanica (Gentianaceae). *American Journal of Botany,* **85**: 811-.

Forrest, C.N., Ottewell, K.M., Whelan, R.J. and Ayre, D.J. 2011. Tests for inbreeding and outbreeding depression and estimation of population differentiation in the bird-pollinated shrub Grevillea mucronulata. *Ann Bot,* **108**: 185-195.

Frankham, R. 2010. Challenges and opportunities of genetic approaches to biological conservation. *Biological Conservation,* **143**: 1919-1927.

Grant, V. 1981. *Plant Speciation*. New York: Columbia University Press.

Hendry, A.P., Bolnick, D.I., Berner, D. and Peichel, C.L. 2009. Along the speciation continuum in sticklebacks. *Journal of Fish Biology,* **75**: 2000-2036.

Hendry, A.P., Nosil, P. and Rieseberg, L.H. 2007. The speed of ecological speciation. *Functional Ecology,* **21**: 455-464.

Hoelzer, G.A., Drewes, R., Meier, J. and Doursat, R. 2008. Isolation-by-Distance and Outbreeding Depression Are Sufficient to Drive Parapatric Speciation in the Absence of Environmental Influences. *PLoS Computational Biology,* **4**: e1000126.

Jump, A.S. and Peñuelas, J. 2006. Genetic effects of chronic habitat fragmentation in a wind-pollinated tree. *Proceedings of the National Academy of Sciences,* **103**: 8096-8100.

Kay, K.M. 2006. Reproductive isolation between two closely related hummingbird pollinated neotropical gingers. *Evolution,* **60**: 538-552.

Kay, K.M. and Sargent, R.D. 2009. The role of animal pollination in plant speciation: integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics,* **40**: 637-656.

Kirkpatrick, M. and Barton, N.H. 1997. Evolution of a species' range. *The American Naturalist,* **150**: 1-23.

Kolb, A. 2005. Reduced reproductive success and offspring survival in fragmented populations of the forest herb Phyteuma spicatum. *Journal of Ecology,* **93**: 1226-1237.

Leblois, R., Estoup, A. and Streiff, R. 2006. Genetics of recent habitat contraction and reduction in population size: does isolation by distance matter? *Molecular Ecology,* **15**: 3601-3615.

Leimu, R., Mutikainen, P., Koricheva, J. and Fischer, M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology,* **94**: 942-952.

Lenormand, T. 2012. From Local Adaptation to Speciation: Specialization and Reinforcement. *International Journal of Ecology,* **2012**: Article ID 508458.

Lowry, D.B., Modliszewski, J.L., Wright, K.M., Wu, C.A. and Willis, J.H. 2008a. The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philosophical Transactions of the Royal Society of London B,* **363**: 3009-30021.

Lowry, D.B., Rockwood, R.C. and Willis, J.H. 2008b. Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution,* **62**: 2196-2214.

Lynch, M. 1991. The genetic interpretation of inbreeding depresison and outbreeding depression. *Evolution,* **45**: 622-629.

Lynch, M., Conery, J. and Burger, R. 1995. Mutational meltdowns in sexual populations. *Evolution,* **49**: 1067-1080.

Mathew, B. 1989. *The Iris*. London: Batsford.

Mayr, E. 1942. *Systematics and the Origin of Species*. New York: Columbia University Press.

Mayr, E. 1947. Ecological Factors in Speciation. *Evolution,* **1**: 263-288.

Morjan, C.L. and Rieseberg, L.H. 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology,* **13**: 1341-1356.

Moyle, L.C. 2006. Correlates of genetic differentiation and isolation by distance in 17 congeneric Silene species. *Molecular Ecology,* **15**: 1067-1081.

Nosil, P., Egan, S.P. and Funk, D.J. 2008. Heterogeneous genomic differentiation between walking-stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. *Evolution,* **62**: 316-336.

Nosil, P., Harmon, L.J. and Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. *Trends in Ecology and Evolution,* **24**: 145-156.

Nosrati, H., Price, A.H. and Wilcock, C.C. 2011. Relationship between genetic distances and postzygotic reproductive isolation in diploid Fragaria (Rosaceae). *Biological Journal of the Linnean Society,* **104**: 510-526.

Paschke, M., Abs, C. and Schmid, B. 2002. Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic Cochlearia bavarica (Brassicaceae). *American Journal of Botany,* **89**: 1250-1259.

Pélabon, C., Carlson, M.L., Hansen, T.F. and Armbruster, W.S. 2005. Effects of crossing distance on offspring fitness and developmental stability in

Dalechampia scandens (Euphorbiaceae). *American Journal of Botany,* **92**: 842-851.

Pinho, C. and Hey, J. 2010. divergence with gene flow: models and data. *Annual Review of Ecology, Evolution and Systematics,* **41**: 215-230.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing.

Ramsey, J., Bradshaw, H.D. and Schemske, D.W. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution,* **57**: 1520-1534.

Rieseberg, L.H., Wood, T.E. and Baack, E.J. 2006. The nature of plant species. *Nature,* **440**: 524-527.

Rundle, H.D. and Nosil, P. 2005. Ecological speciation. *Ecology Letters,* **8**: 336-352.

Sambatti, J.B.M., Strasburg, J.L., Ortiz-Barrientos, D., Baack, E.J. and Rieseberg, L.H. 2012. Reconnciling extremely strong barriers with high levels of gene exchange in annual sunflowers. *Evolution,* **66**: 1459-1473.

Sapir, Y., Shmida, A. and Fragman, O. 2003. Constructing Red Numbers for endangered plant species - Israeli flora as a test case. *Journal for Nature Conservation,* **11**: 91-107.

Sapir, Y., Shmida, A., Fragman, O. and Comes, H.P. 2002. Morphological variation of the *Oncocyclus* Irises (*Iris*: Iridaceae) in the Southern Levant. *Botan J Linn Soc,* **139**: 369-382.

Sapir, Y., Shmida, A. and Ne'eman, G. 2005. Pollination of the *Oncocyclus* irises (*Iris*: Iridaceae) by night-sheltering male bees. *Plant Biology,* **7**: 417-424.

Sapir, Y., Shmida, A. and Ne'eman, G. 2006. Morning floral heat as a reward to the pollinators of the *Oncocyclus* irises. *Oecologia,* **147**: 53-59.

Saunders, D.A., Hobbs, R.J. and Margules, C.R. 1991. Biological Consequences of Ecosystem Fragmentation: A Review. *Conservation Biology,* **5**: 18-32.

Schleuning, M., Templin, M., Huamán, V., Vadillo, G.P., Becker, T., Durka, W., Fischer, M. and Matthies, D. 2011. Effects of Inbreeding, Outbreeding, and Supplemental Pollen on the Reproduction of a Hummingbird-pollinated Clonal Amazonian Herb. *Biotropica,* **43**: 183-191.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science,* **323**: 737-741.

Schmidt, K. and Jensen, K. 2000. Genetic structure and AFLP variation of remnant populations in the rare plant Pedicularis palustris (Scrophulariaceae) and its relation to population size and reproductive components. *American Journal of Botany,* **87**: 678-689.

Scopece, G., Musacchio, A., Widmer, A. and Cozzolino, S. 2007. Patterns of reproductive isolation in mediterranean deceptive orchids. *Evolution,* **61**: 2623-2642.

Segal, B., sapir, Y. and Carmel, Y. 2007. Fragmentation and pollination crisis in the self-incompatible *Iris bismarckiana* (Iridaceae), with implications for conservation. *Israel Journal of Ecology and Evolution,* **52**: 111-122.

Servedio, M.R. and Noor, M.A.F. 2003. The role of reinforcement in speciation: theory and data. *Annual Review of Ecology, Evolution and Systematics,* **34**: 339-364.

Severns, P., Liston, A. and Wilson, M. 2011. Habitat fragmentation, genetic diversity, and inbreeding depression in a threatened grassland legume: is genetic rescue necessary? *Conservation Genetics,* **12**: 881-893.

Shi, X., Michaels, H. and Mitchell, R. 2005. Effects of self-pollination and maternal resources on reproduction and offspring performance in the wild lupine, *Lupinus perennis* (Fabaceae). *Sexual Plant Reproduction,* **18**: 55-64.

Shmida, A. and Pollak, G. 2007. *Red Data Book: Endangered Plants of Israel*, Vol. 1. Jerusalem: Nature-Parks Authority Press.

Smadja, C.M. and Butlin, R.K. 2011. A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology,* **20**: 5123-5140.

Smith, R.A. and Rausher, M.D. 2007. Close clustering of anthers and stigma in Ipomoea hederacea enhances prezygotic isolation from Ipomoea purpurea. *New Phytologist,* **173**: 641-647.

Sobrevila, C. 1988. Effects of distance between pollen donor and pollen recipient on fitness components in *Espeletia schultzii*. *American Journal of Botany,* **75**: 701-724.

Stacy, E.A. 2001. Cross-fertility in two tropical tree species: evidence of inbreeding depression within populations and genetic divergence among populations. *American Journal of Botany,* **88**: 1041-1051.

Stuurman, J., Hoballah, M.E., Broger, L., Moore, J., Basten, C. and Kuhlemeier, C. 2004. Dissection of Floral Pollination Syndromes in Petunia. *Genetics,* **168**: 1585-1599.

Sun, S. and Cheptou, P.-O. 2012. Life-history traits evolution across distribution ranges: how the joint evolution of dispersal and mating system favor the evolutionary stability of range limits? *Evolutionary Ecology,* **26**: 771-778.

Tabarelli, M. and Gascon, C. 2005. Lessons from Fragmentation Research: Improving Management and Policy Guidelines for Biodiversity Conservation. *Conservation Biology,* **19**: 734-739.

Van Geert, A., Van Rossum, F. and Triest, L. 2010. Do linear landscape elements in farmland act as biological corridors for pollen dispersal? *Journal of Ecology,* **98**: 178-187.

Volis, S. 2007. Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology,* **21**: 381-393.

Volis, S., Bohrer, G., Oostermeijer, G. and Van Tienderen, P. 2005. Regional Consequences of Local Population Demography and Genetics in Relation to Habitat Management in Gentiana pneumonanthe. *Conservation Biology,* **19**: 357-367.

Waser, N.M. and Price, M.V. 1989. Optimal outcrossing in *Ipomopsis aggregata* - seed set and offspring fitness. *Evolution,* **43**: 1097-1109.

Waser, N.M. and Williams, C.F. 2001. Inbreeding and Outbreeding. In *Evolutionary Ecology. Concepts and Case Studies* (C.W. Fox, D.A. Roff and D.J. Fairbaine, eds), pp. 84-96. Oxford: Oxford University Press.

Wilcox, B.A. and Murphy, D.D. 1985. Conservation strategy: the effects of fragmentation on extinction. *The American Naturalist,* **125**: 879-887.

Willi, Y., Van Buskirk, J., Schmid, B. and Fischer, M. 2007. Genetic isolation of fragmented populations is exacerbated by drift and selection. *Journal of Evolutionary Biology,* **20**: 534-542.

Tables

Table 1. Analyses of variance of four components of reproductive isolation among populations in *Iris atropurpurea*. Pollen donor population effect is nested within region effect (within population, near populations and far populations), which, in turn, is nested within experimental site (NET or SHF). Seed-set was log-transformed and germination was arc-sin transformed to improve normality. Significant effects are in bold.

	Source	DF	SS	F	Sig.
Fruit-set	**Population**	1	0.58	6.08	P=0.014
	Population (region)	4	0.78	2.03	P=0.090
	Population [region (donor)]	12	1.34	1.16	P=0.310
	Residuals	343	32.87		
Seed-set	Population	1	1.47	0.69	P=0.407
	Population (region)	4	21.67	2.54	P=0.040
	Population [region (donor)]	12	39.90	1.56	P=0.103
	Residuals	266	566.85		
Germination	Population	1	0.004	0.86	P=0.356
	Population (region)	4	0.251	12.60	P<0.001
	Population [region (donor)]	10	0.266	5.34	P<0.001
	Residuals	74	0.369		
Seedling survival	**Population**	1	0.81	3.90	P=0.049
	Population (region)	4	0.41	0.49	P=0.747
	Population [region (donor)]	12	9.64	3.85	P<0.001
	Residuals	428	89.34		

Table 2. Values of four components of reproductive isolation in crosses to plants in Netanya (NET). N is the number of flowers used in the population that received pollen from each donor population. Fruit-set is the fraction of fruits produced from flowers. Seed-set is average number of seeds produced per flower. Germination is fraction of seeds germinated in two years. Seedling survival is fraction of seedlings from the germinating seeds that survived one year after planting in natural conditions. F values and significance are from ANOVA with binomial distribution for fruit-set and Poisson distribution for seed-set. Population abbreviations: NET – Netanya (within population) ; DOR – Dora dunes; PLG – Poleg reserve; YQM – Yaqum; SHF – Shafdan; IRE – Iris reserve; YVN – Yavne.

	NET	DOR	PLG	YQM	SHF	IRE	YVN		
Distance (Km)	0	0.45	2.6	4	37.5	40	45.6		
N	68	10	26	14	14	13	7	F	Sig.
Fruit-set	0.84	0.90	0.96	0.79	0.86	0.69	0.71	1.03	P=0.38
Seed-set	28.9	32.1	39.3	37.0	31.9	21.7	32.6	1.01	P=0.42
Germination	0.19	0.21	0.14	0.17	0.26	0.29	0.20	3.64	P=0.007
Seedling survival	0.65	0.3	1.0	0.75	0.39	0.67	0.6	4.83	P<0.001

Table 3. Values of four components of reproductive isolation in crosses to plants in Shafdan (SHF). N is the number of flowers used in the population that received pollen from each donor population. Fruit-set is the fraction of fruits produced from flowers. Seed-set is average number of seeds produced per flower. Germination is fraction of seeds germinated in two years. Seedling survival is fraction of seedlings from the germinating seeds that survived one year after planting in natural conditions. F values and significance are from ANOVA with binomial distribution for fruit-set and Poisson distribution for seed-set. Population abbreviations: SHF – Shafdan (within population) ; IRE – Iris reserve; NSZ – Nes Ziona; RLZ – Rishon Lezion; YVN – Yavne; ASH – Ashdod; TLV – Tel Aviv; NET – Netanya; NTF – Netanya forest; KDM – Kadima; HDR – Hadera.

	SHF	IRE	NSZ	RLZ	YVN	ASH	TLV	NET	NTF	KDM	HDR		
Distance (Km)	0	2.6	3.8	4.4	8.3	14.3	19.9	37.5	40.4	42.4	58.5		
N	97	6	6	7	18	12	6	19	4	5	29	F	Sig.
Fruit-set	0.88	1.0	1.0	0.86	1.0	1.0	1.0	0.95	1.0	0.60	1.0	1.84	P=0.056
Seed-set	28.7	47.0	47.7	44.2	43.3	45.2	29.0	41.3	13.5	0.7	44.8	1.84	P=0.059
Germination	0.34	0.22	0.16	0.26	0.09	0.23	0.04	0.24			0.25	9.42	P<0.001
Seedling survival	0.70	0.69	0.67	1.0	0.57	0.75	1.0	0.55			0.85	1.92	P=0.057

Figures

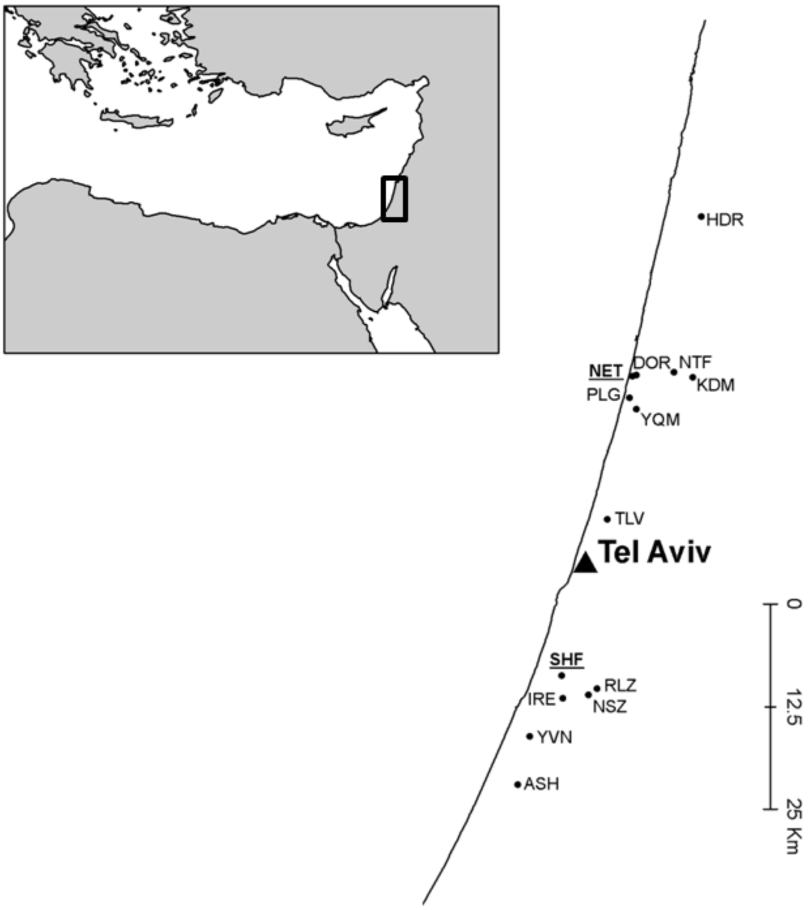

Figure 1. Map of *Iris atropurpurea* populations used in the cross experiment. For population abbreviations see Table 2 and Table 3.

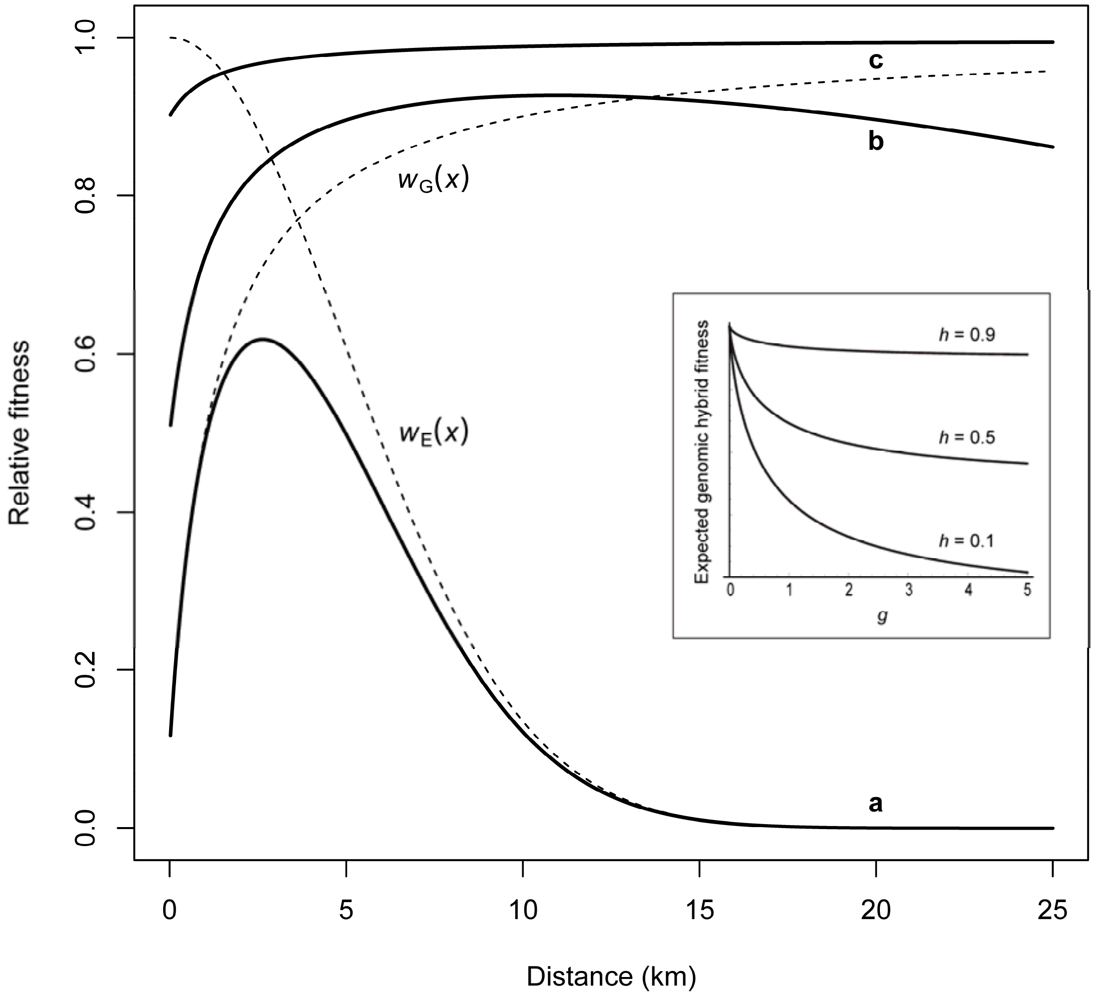

Figure 2. Relative hybrid fitness $w = w_G w_E$ as a function of crossing distance for various values of the parameters: environmental gradient slope s, relative genomic fitness of local crosses h, and distance g where w_G attains half its maximum value. For the x-axis, the dimensionless distances were translated into actual distances by assuming a pollination distance of $250\,\mathrm{m}$. a) $h = 0.1$, $s = 0.1$ (the dashed lines indicate the corresponding component functions for this case); b) $h = 0.5$, $s = 0.01$; c) $h = 0.9$, $s = 0.001$; a)+b)+c) $g = 5$. Inset: Genomic offspring fitness expected under natural pollination conditions, $\langle w_G \rangle = \int w_G(x)\exp(-x^2/2)\mathrm{d}x$ with an assumed pollination kernel $\exp(-x^2/2)$, as a function of g for various values of h.

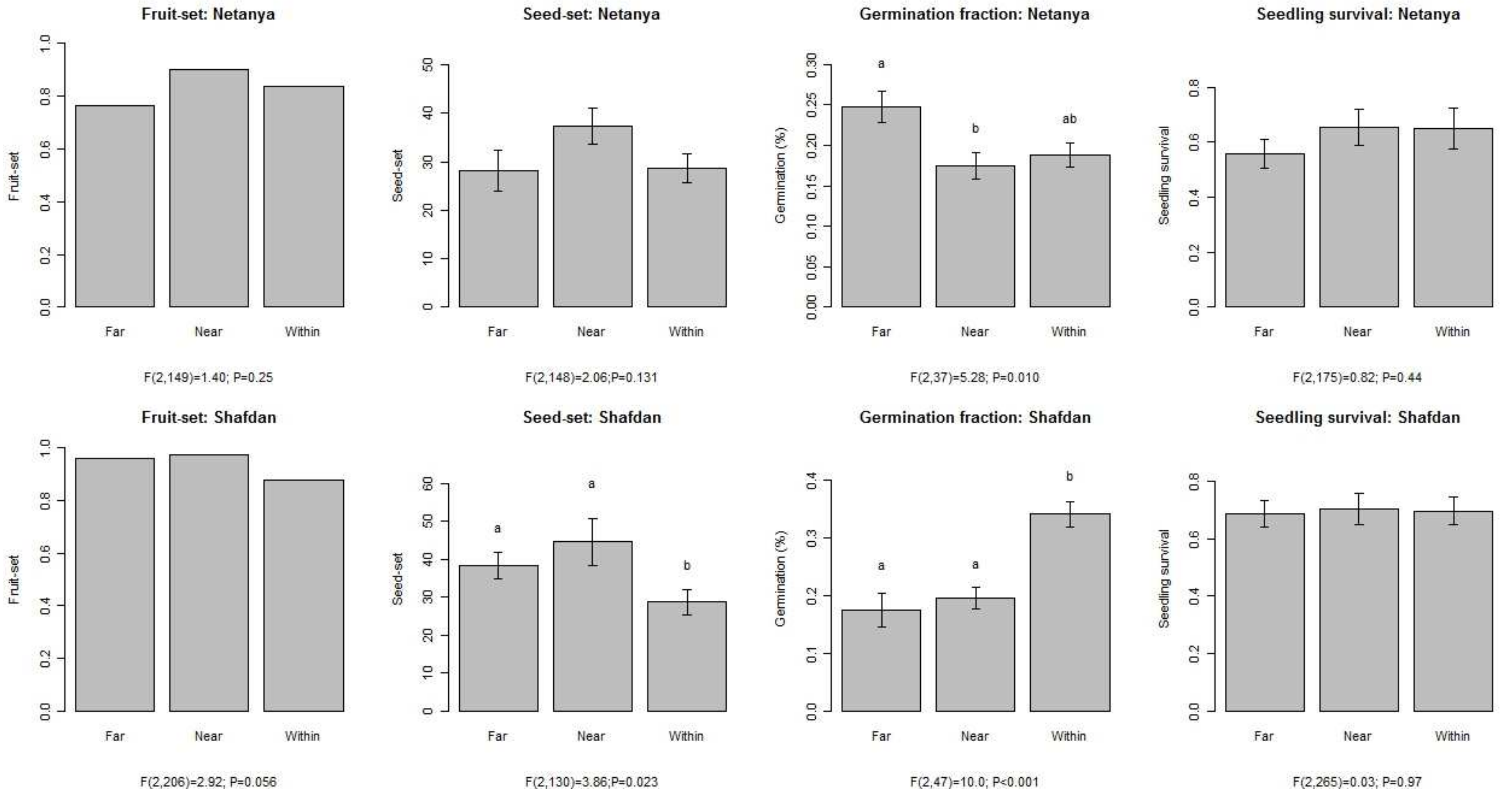

Figure 3. Differences in fitness components between crosses within populations and with near or far populations in two *Iris atropurpurea* populations – Netanya (top) and Shafdan (bottom). Bars are means (± SE). Same letters above bars

denote values not significantly different (Contrast analyses; $P>0.05$). A contrast analysis was performed only if a significant effect of cross category was found.

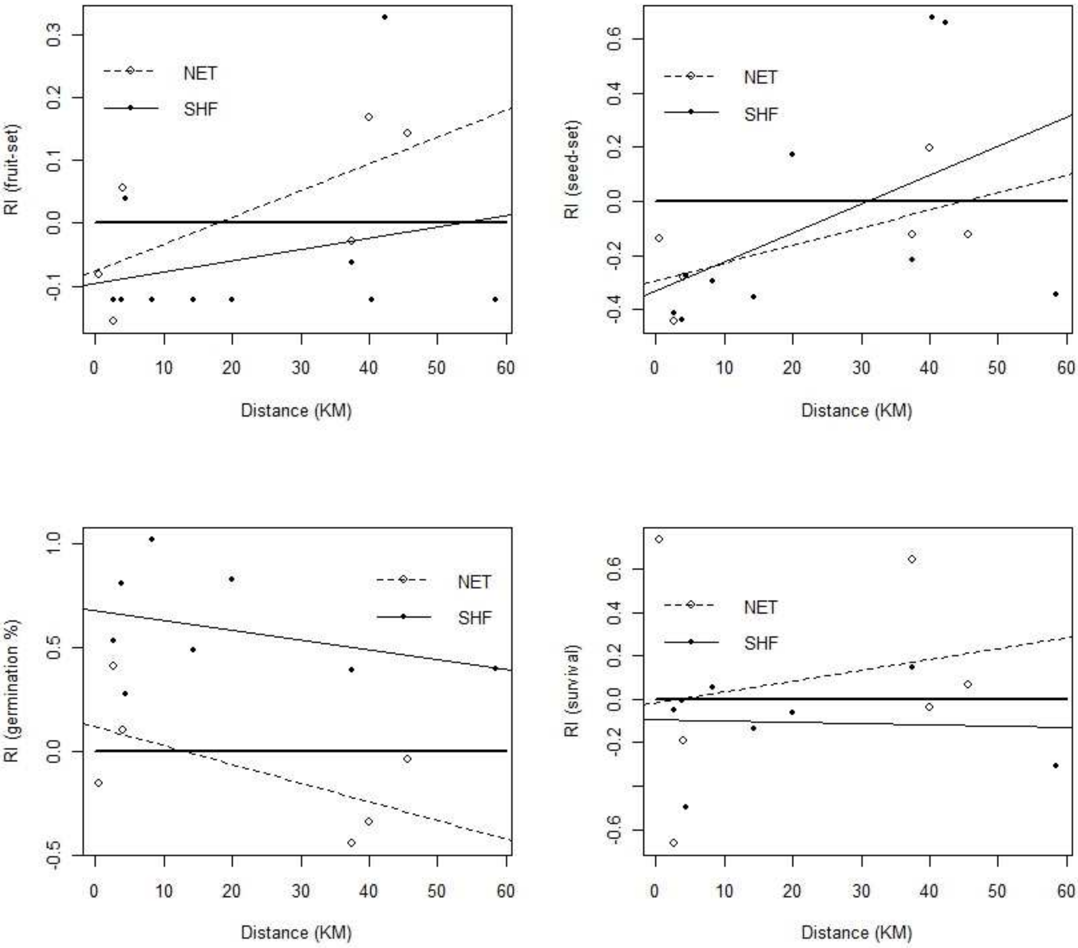

Figure 4. Absolute reproductive isolation values of four fitness components as a function of distance, in two *Iris atropurpurea* populations. The bold horizontal line in each graph denotes zero reproductive isolation.

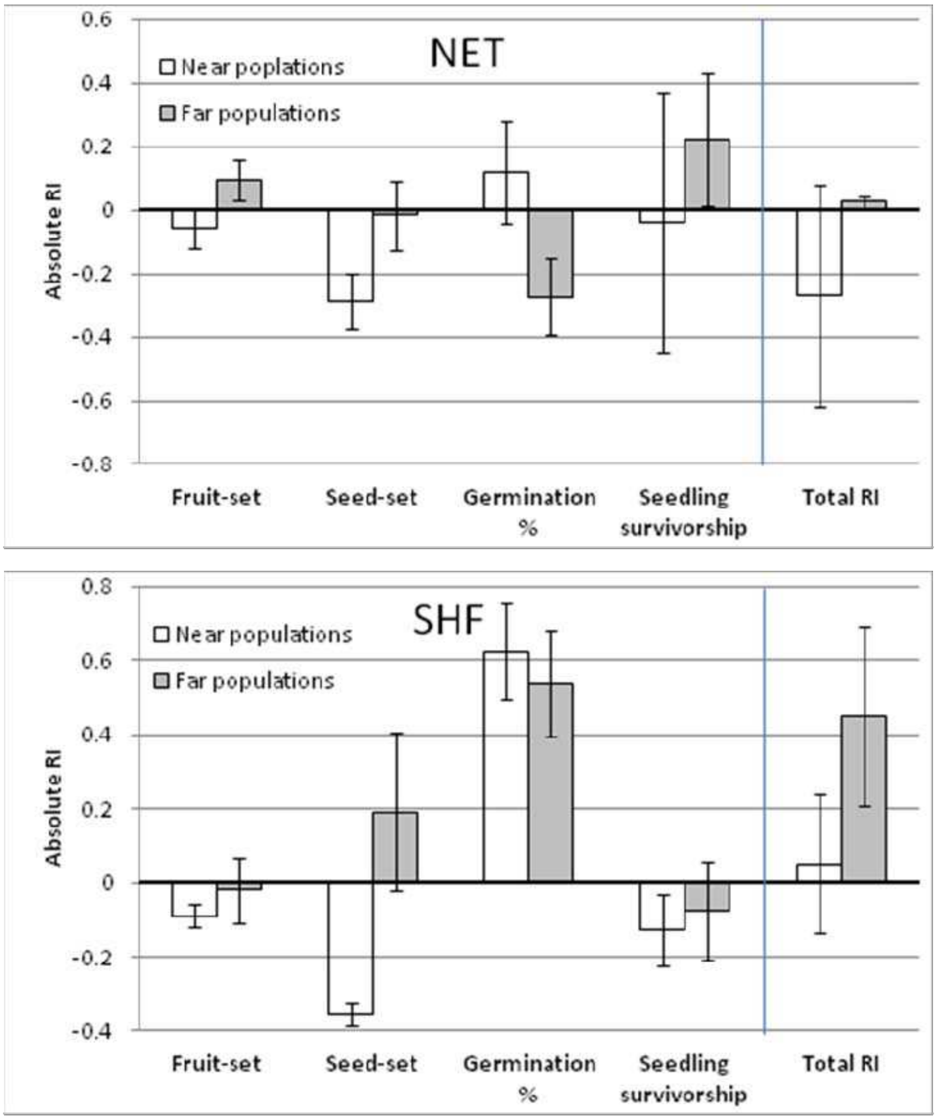

Figure 5. Absolute reproductive isolation values of four fitness components and total reproductive isolation for crosses with near and far pollen-donor populations in two *Iris atropurpurea* populations. Bars represent mean (± SE) of reproductive isolation values for the populations of each region.

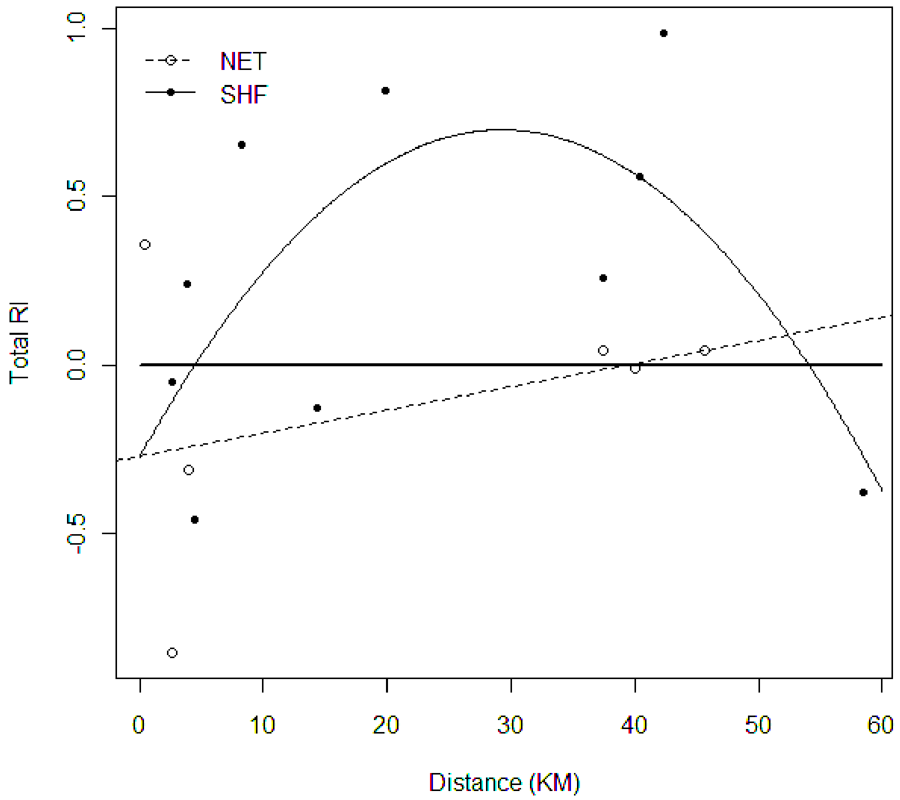

Figure 6. Total reproductive isolation values as a function of distance in two *Iris atropurpurea* populations. Bold horizontal line denotes zero reproductive isolation. The quadratic regression line for SHF population is marginally significant (P=0.053). Linear slope for NET population is not significantly different from zero (P=0.49). Note that although similarity to the theoretical pattern (Figure 2), it is the reciprocity of hybrid fitness, and thus the pattern is the opposite.