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Testing for beneficial reversal of dominance during salinity shifts in the invasive copepod *Eurytemora affinis*, and implications for the maintenance of genetic variation

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Maintenance of genetic variation at loci under selection has profound implications for adaptation under environmental change. In temporally and spatially varying habitats, non-neutral polymorphism could be maintained by heterozygote advantage across environments (marginal overdominance), which could be greatly increased by beneficial reversal of dominance across conditions. We tested for reversal of dominance and marginal overdominance in salinity tolerance in the saltwater-to-freshwater invading copepod *Eurytemora affinis*. We compared survival of F1 offspring generated by crossing saline and freshwater inbred lines (between-salinity F1 crosses) relative to within-salinity F1 crosses, across three salinities. We found evidence for both beneficial reversal of dominance and marginal overdominance in salinity tolerance. In support of reversal of dominance, survival of between-salinity F1 crosses was not different from that of freshwater F1 crosses under freshwater conditions and saltwater F1 crosses under saltwater conditions. In support of marginal overdominance, between-salinity F1 crosses exhibited significantly higher survival across salinities relative to both freshwater and saltwater F1 crosses. Our study provides a rare empirical example of complete beneficial reversal of dominance associated with environmental change. This mechanism might be crucial for maintaining genetic variation in salinity tolerance in *E. affinis* populations, allowing rapid adaptation to salinity changes during habitat invasions.

KEY WORDS: **Adaptation, balancing selection, genetic polymorphism, invasive species, marginal overdominance, temporally varying environment.**

The performance of a population under selection depends on levels of genetic variation underlying relevant phenotypic traits (Crow and Kimura 1970). When populations are invading novel environments, adaptation at critical traits is often required for the populations to survive and persist (Reznick and Ghalambor 2001; Lee et al. 2003,2007,2011; Phillips et al. 2006; Simons 2007; Keller and Taylor 2008; Lee and Gelembiuk 2008; Prentis et al. 2008; Nielsen et al. 2012). Given the waiting time required for de novo mutations, it is thought that rapid adaptation during invasions relies predominantly on standing genetic variation (Barrett and Schluter 2008; Lee and Gelembiuk 2008; Prentis et al. 2008). Theoretical studies indicate that high standing

Tables S1-S3 have been replaced with corrected versions as of 29 January 2015.

genetic variation in source populations greatly facilitates adaptation into novel stressful habitats (Gomulkiewicz et al. 1999; Boulding and Hay 2001; Holt et al. 2003; Innan and Kim 2004; Kim and Gulisija 2010; Holt and Barfield 2011). Yet, the details of how non-neutral polymorphism is generated and maintained within populations in nature remain inadequately understood (Turelli and Barton 2004; Mitchell-Olds et al. 2007). This study tests for the presence of an underlying mechanism that potentially acts to facilitate the maintenance of genetic variation within populations, particularly for traits under selection during ecological invasions. At a locus under selection, observed levels of variation are attributable to two major mechanisms: mutation-selection balance (Lande 1975; Houle et al. 1996) and balancing selection (Gillespie and Turelli 1989; Turelli and Barton 2004). The relative importance of these two mechanisms remains unclear (Houle et al. 1996; Turelli and Barton 2004, Mitchell-Olds et al. 2007). Balancing selection tends to lead to the maintenance of alleles of intermediate frequencies, whereas mutation-selection balance tends to lead to a prevalence of rare alleles (Turelli and Barton 2004). Balancing selection refers to any type of selection that maintains genetic variation, such as overdominance, frequency-dependent selection, and antagonistic selection. In particular, alleles that are subject to opposing selection (e.g., that are beneficial in some environmental contexts and detrimental in others) could be maintained in a population by antagonistic selection between spatially or temporally varying environments (Levene 1953; Haldane and Jayakar 1963; Wallace 1968; Hedrick 1974, 1976, 1986; Felsenstein 1976; Curtsinger et al. 1994; Dean 2005; Epinat and Lenormand 2009; Carter and Nguyen 2011; Connallon and Clark 2012a). Temporally varying selection is of particular interest as a mechanism that maintains variation, because a large number of successful invaders appear to have originated from disturbance-prone temporally varying environments, more than what might be expected due to transport opportunity alone (Lee and Gelembiuk 2008).

In diploid organisms, balancing selection via marginal overdominance can maintain polymorphism for alleles that are antagonistically selected across spatially and temporally varying environments. Marginal overdominance refers to the case when heterozygotes possess higher mean fitness across environments relative to that of any homozygote, even when fitness of the heterozygotes does not exceed that of the best-fit homozygote within any specific environment (Wallace 1968). Marginal overdominance could operate over both temporal and spatial scales. Geometric mean overdominance is sufficient for maintaining protected polymorphisms under a basic model of temporal variation in fitness with random mating (Haldane and Jayakar 1963; Gillespie 1973, 1974, 1998; Hedrick 1976, 1986, 2002, 2005, 2006; Hoekstra et al. 1985). On the other hand, harmonic mean overdominance is sufficient for maintaining protected polymorphism under a basic model of spatial variation in fitness with random mating (Levene 1953). Conditions for harmonic mean overdominance are less stringent than for geometric mean overdominance, because the harmonic mean is always less than or equivalent to the geometric mean (and the geometric mean is always less than or equivalent to the arithmetic mean) (Felsenstein 1976). Thus, conditions for maintaining protected polymorphisms under spatial variation appear less stringent than under temporal variation. Additionally, in the presence of recurrent mutation, antagonistic selection can inflate genetic variance to a much greater extent and over a far wider parameter range than could be produced by protected polymorphisms alone (Bürger and Gimelfarb 2002; Connallon and Clark 2012b; Delph and Kelly 2014).

Under antagonistic selection, beneficial reversal of dominance can greatly increase the magnitude of marginal overdominance (i.e., by increasing the mean fitness of heterozygotes) (Kidwell et al. 1977; Curtsinger et al. 1994; Fry 2010; Connallon and Clark 2012b). Beneficial reversal of dominance refers to the case where dominance switches at a locus across distinct traits (e.g., fitness in different environments) such that an allele is always dominant for the trait where it is beneficial and recessive where harmful. For example, in the context of a metabolic pathway, such a switching of dominance across environments might arise due to the more-fit allele in a given environment compensating for the lower function of the allele maladapted to that environment (Wright 1934; Kacser and Burns 1981). Beneficial reversal of dominance greatly increases the parameter range under which polymorphism is favored and the efficiency with which antagonistically selected alleles are maintained (Curtsinger et al. 1994; Connallon and Clark 2012b). Without beneficial reversal of dominance, the strength of balancing selection for a pair of antagonistically selected alleles is on the order of $N_e s_1 s_2$, whereas with beneficial reversal of dominance, the strength of balancing selection is on the order of $N_e(s_1 + s_2)(1 - 2h)$ (where N_e is effective population size, s_1 and s_2 are selection coefficients, and h is a dominance parameter), which might be orders of magnitude greater (Connallon and Clark 2012b). Masking of conditionally deleterious alleles in heterozygotes can thus dramatically strengthen balancing selection generated by marginal overdominance.

Yet, despite the potential for beneficial reversal of dominance to facilitate adaptation to changing environments, empirical studies demonstrating beneficial reversal of dominance are still rare (Caspari 1950; Kohn et al. 2003; Roux et al. 2004; López et al. 2010; Hund et al. 2012) (see Discussion for details). Many studies have focused either on testing for marginal overdominance or on detecting allele frequency fluctuations without testing specifically for beneficial reversal of dominance (Wills 1975; Watt 1983; Hedrick 2002; Mojica et al. 2012; Bergland et al. 2014). Thus,

the goal of this study was to test explicitly whether beneficial reversal of dominance for salinity tolerance is operating in the copepod *Eurytemora affinis*. This copepod has invaded freshwater from saline habitats multiple times independently from genetically distinct sources (Lee 1999). Interestingly, saline (brackish) populations of *E. affinis* that are able to invade freshwater habitats appear to originate from habitats marked by large seasonal fluctuations in salinity (though never completely fresh), whereas saline populations of *E. affinis* that reside in environments with less variation in salinity have not invaded (Lee 1999; Winkler et al. 2008). Thus, an evolutionary history in fluctuating environments potentially corresponds to invasiveness in this species (Lee and Gelembiuk 2008).

Beneficial reversal of dominance in its most extreme form would result in freshwater tolerance being completely dominant under freshwater conditions and saltwater tolerance being completely dominant under saline conditions. The less-fit alleles would always be masked from selection in the heterozygous state, preventing erosion of genetic variation for salinity tolerance in both saltwater and freshwater environments (Wallace 1968; Hoekstra et al. 1985; Curtsinger et al. 1994). Such reversal of dominance would explain results from previous studies on *E. affinis*, which found that alleles favoring high-salinity tolerance were apparently maintained in a decades-old freshwater population and alleles favoring freshwater tolerance were apparently retained in a saline population, despite the presence of trade-offs between fresh and higher salinity tolerance (Lee et al. 2003, 2007). Moreover, temporally varying antagonistic selection in the presence of reversal of dominance could greatly inflate genetic variation for salinity tolerance in the native, fluctuating estuarine environment. Reversal of dominance should (by rendering any freshwater-beneficial alleles dominant in freshwater) increase initial rates of survival for stocks of *E. affinis* transplanted from the native estuarine environment to a freshwater environment. Most importantly, maintenance of high levels of genetic variation for salinity tolerance in the native estuarine population would increase the ability of invading freshwater-transplanted stocks to rapidly adapt to the novel freshwater environment (Barrett and Schluter 2008; Lee and Gelembiuk 2008).

To test for reversal of dominance, and consequently marginal overdominance, we compared survival across salinities of F1 crosses between inbred lines generated from saline and freshwater populations (salt × fresh), relative to control F1 crosses made between saline (salt × salt) or between freshwater lines (fresh × fresh). Support for beneficial reversal of dominance would be evident if survival of F1 crosses between the saltwater and freshwater inbred lines were equivalent to that of saltwater F1 crosses (salt × salt) under saltwater conditions (15 PSU), and to that of freshwater F1 crosses (fresh × fresh) under freshwater conditions (0 PSU). Relatively high survival of saltwater × freshwater F1 crosses across all environments, resulting in the average advantage of the heterozygotes over homozygotes, would provide evidence for marginal overdominance.

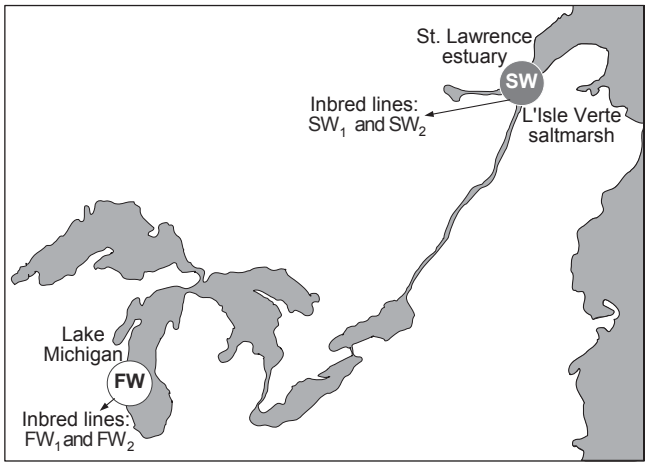

Figure 1. Populations of the copepod *E. affinis* used in this study. The ancestral saltwater population (SW) from Baie de L'Isle Verte salt marsh in the St. Lawrence estuary was used to create two independent saltwater inbred lines (SW_1 and SW_2). The derived freshwater population (FW) from Lake Michigan was established by a recent invasion from the St. Lawrence estuary into the Great Lakes around 1958 (Engel 1962; Lee 1999). Two independent freshwater inbred lines (FW_1 and FW_2) were generated from the Lake Michigan population.

Material and Methods

EXPERIMENTAL INBRED LINES

To test for the presence of beneficial reversal of dominance (and marginal overdominance), we crossed inbred lines derived from saline and freshwater populations of *E. affinis*, and compared survival of these between-salinity F1 crosses to that of within-salinity F1 crosses (see next paragraph for details on experimental design). We generated four inbred lines independently, two each from two populations of *E. affinis* through full-sibling mating for 30 generations (2.5 years). Two independent saltwater inbred lines (SW_1 and SW_2) were derived from the ancestral saline population in Baie de L'Isle Verte, St. Lawrence marsh, Quebec, Canada (Fig. 1; 48°00′14″N, 69°25′31″W), whereas two freshwater inbred lines (FW_1 and FW_2) were derived from the freshwater invading population in Lake Michigan at Racine Harbor, Wisconsin, USA (Fig. 1; 42°43′46″N, 87°46′44″W). The two saltwater inbred lines were generated and reared at their native salinity of 15 PSU (PSU ≈ parts per thousand salinity), whereas the two freshwater inbred lines were generated and reared in Lake Michigan water (0 PSU, conductivity ≈ 300 μS/cm).

Table 1. Full diallel mating scheme (Lynch and Walsh 1998) of four independent inbred lines of *E. affinis* resulting in 16 different F1 offspring used in this study (the first inbred line denotes the male parent).

INDEPENDENT INBRED LINES (MALES) \ INDEPENDENT INBRED LINES (FEMALES)	FW_1	FW_2	SW_1	SW_2
FW_1	$FW_1 \times FW_1$	$FW_1 \times FW_2$	$FW_1 \times SW_1$	$FW_1 \times SW_2$
FW_2	$FW_2 \times FW_1$	$FW_2 \times FW_2$	$FW_2 \times SW_1$	$FW_2 \times SW_2$
SW_1	$SW_1 \times FW_1$	$SW_1 \times FW_2$	$SW_1 \times SW_1$	$SW_1 \times SW_2$
SW_2	$SW_2 \times FW_1$	$SW_2 \times FW_2$	$SW_2 \times SW_1$	$SW_2 \times SW_2$

FW_1 and FW_2—two independent freshwater inbred lines derived from the freshwater Lake Michigan population (Fig. 1, FW)
SW_1 and SW_2—two independent saltwater inbred lines derived from the saline L'Isle Verte population (Fig. 1, SW).
(a) Matings within parental inbred lines are on the diagonal. (b) Within-salinity F1 crosses (light gray cells, FW-F1 and SW-F1) are crosses between two independent inbred lines derived from the same population. (c) Between-salinity crosses F1 (dark gray cells, SW × FW-F1) are crosses between saline and freshwater inbred lines.

EXPERIMENTAL DESIGN

To test for the presence of beneficial reversal of dominance and marginal overdominance, we performed crosses between and within the saline and freshwater inbred lines and compared their survival across salinities. Specifically, we performed a full diallel mating scheme (Table 1) to generate three types of F1 offspring: (a) matings within parental inbred lines (Fig. 2B-(a); Table 1, diagonal) as controls, (b) within-salinity F1 crosses, namely matings between inbred lines derived independently from the same population (i.e., Fig. 2B-(b); Table 1, light gray cells, crosses between FW_1 and FW_2 and between SW_1 and SW_2; denoted hereon as "SW-F1" and "FW-F1"), to account for effects of heterosis that might arise from mating between genetically distinct inbred lines, and (c) between-salinity F1 crosses (Fig. 2B-(c), Table 1, dark gray cells, denoted hereon as "SW × FW-F1"), referring to matings performed between saline (SW_1 or SW_2) and freshwater (FW_1 or FW_2) inbred lines. The F1 offspring were then reared across three salinities (0, 2.5, 15 PSU) to measure survival and infer changes in dominance of salinity tolerance across salinities (Fig. 2C, described below).

The between-salinity F1 crosses (c, above; Fig. 2B-(c)) would be heterozygous at loci that would confer saline or freshwater tolerance. Comparing survival of these between-salinity crosses to survival of the parental inbred lines (a, above; Fig. 2B-(a)) and to that of the within-salinity F1 crosses (b, above; Fig. 2B-(b)) allowed us to assess the switching of dominance in salinity tolerance between saline and freshwater habitats. This comparison allowed us to observe whether the heterozygotes (i.e., between-salinity SW × FW-F1 crosses) exhibited survival that was not significantly different from that of the more-fit homozygote in each habitat. If this were the case, the more-fit allele of the heterozygote would be exhibiting dominance in each habitat (see below).

We created the within-salinity F1 crosses (mating between inbred lines derived independently from the same population; Fig. 2B-(b), SW-F1 and FW-F1 crosses) to account for increased survival that might arise from heterosis (hybrid vigor). The inbred

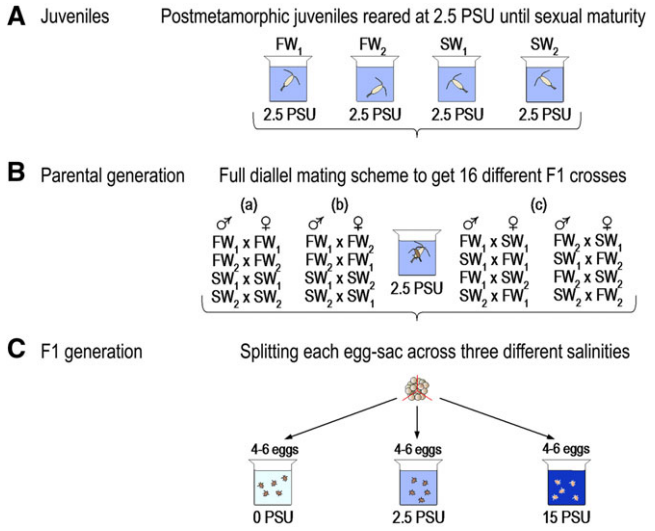

Figure 2. **Experimental design to test for reversal of dominance and marginal overdominance in salinity tolerance for the copepod *E. affinis*. (A) Juveniles from all four saline and freshwater parental inbred lines (FW_1, FW_2, SW_1, and SW_2) were gradually transferred to a common salinity of 2.5 PSU, and reared at this salinity until they became sexually mature. (B) Upon reaching sexual maturity, 16 mating combinations were formed (as shown in Table 1) to obtain three different types of F1 offspring: (a) parental inbred lines (SW_1, SW_2, FW_1, and FW_2), (b) reciprocal within-salinity F1 crosses (SW-F1 and FW-F1), and (c) reciprocal between-salinity F1 crosses (SW × FW-F1). (C) After successful mating, egg sacs (F1 offspring) were removed from females and each egg sac was split across three salinities (0, 2.5, and 15 PSU) and reared until adulthood to measure survival and infer changes in dominance of salinity tolerance across salinities. The common-garden experiment was conducted in two blocks at two different time periods.**

lines that were independently derived from the same populations are likely to have become fixed for different recessive or partially recessive deleterious alleles at some loci across their genomes. F1 crosses between inbred lines fixed for deleterious alleles at different loci would display heterosis due to masking of expression

of deleterious alleles in the heterozygous state (Charlesworth and Charlesworth 1987, 1999). Therefore, within-salinity F1 crosses (Fig. 2B-(b), SW-F1 and FW-F1) could be used to account for heterosis that might arise from crossing genetically distinct inbred lines. Thus, higher survival of the FW-F1 and SW-F1 crosses relative to the parental inbred lines (Fig. 2B-(a), matings within SW_1, SW_2, FW_1, and FW_2) would be the result of heterosis.

Given that the within-salinity F1 crosses (Fig. 2B-(b), SW-F1 and FW-F1) could account for heterosis, we compared their survival to that of the between-salinity F1 crosses (Fig. 2B-(c), SW × FW-F1) to test for reversal of dominance and marginal overdominance. Support for beneficial reversal of dominance would be evident if the between-salinity F1 crosses (SW × FW-F1) showed no significant difference in survival relative to that of within-saltwater crosses (SW-F1) under saltwater conditions and relative to that of within-freshwater crosses (FW-F1) under freshwater conditions. Such a result would indicate the switching of dominance in salinity tolerance between saline and freshwater conditions. On the other hand, significantly higher survival of the between-salinity F1 crosses (SW × FW-F1) relative to FW-F1 crosses under freshwater conditions and relative to SW-F1 crosses under saltwater conditions would provide support for overdominance at the salinity tolerance loci. To test for the presence of marginal overdominance, we compared marginal survival (arithmetic mean survival across all three salinities, 0, 2.5, and 15 PSU) of the between-salinity F1 crosses (SW × FW-F1) relative to survival of both within-salinity F1 crosses (SW-F1 and FW-F1). Marginal overdominance would be evident if marginal survival of the SW × FW-F1 crosses were significantly higher than that of the SW-F1 and FW-F1 crosses.

PERFORMING THE COMMON-GARDEN EXPERIMENT

To assess the presence of reversal of dominance and marginal overdominance, we reared the different types of F1 crosses (Fig. 2B) under three common-garden salinities and compared their survival at the different salinities (Fig. 2C). We conducted the common-garden experiments under controlled standard conditions, in a 13°C environmental chamber on a 15L:9D photoperiod. To ensure that individuals were virgins prior to crossing, we isolated ca. 200 postmetamorphic juveniles from each inbred line and placed them individually into 20 mL scintillation vials filled with 10 mL of 2.5 PSU water (Fig. 2A). To avoid osmotic shock, we gradually transferred juveniles from all four inbred lines (SW_1, SW_2, FW_1, and FW_2) to a common salinity of 2.5 PSU, and then reared them at this salinity until they reached sexual maturity (Fig. 2A). We reared juveniles at this salinity because it was the least stressful common environment for both the saline and freshwater populations. We started the experiment with postmetamorphic juveniles to avoid imposing selection (on any residual genetic variance) in response to salinity in the parental inbred lines prior to mating. Prior experiments revealed that most mortality in response to non-native salinities occurs before metamorphosis, and that the copepods are less sensitive to osmotic stress following metamorphosis to the juvenile (copepodid) stage (Lee et al. 2003, 2007). After 8–10 days of developmental acclimation at 2.5 PSU (i.e., when juveniles became sexually mature adults), we performed a full diallel mating scheme as described in the previous section (Table 1 and Fig. 2B). Following successful mating, we removed egg sacs (F1 offspring) from females and split each egg sac across the three treatment salinities (0, 2.5, and 15 PSU) into separate vials (4–6 eggs per vial) and reared them until adulthood (Fig. 2C). We recorded hatching and survival every second day.

We conducted the common-garden experiment in two blocks at different time periods, consisting of 3–8 replicate clutches per cross in the first block (July to October 2010) and 5–11 replicate clutches per cross in the second block (February to May 2011). In total we used 184 clutches, with 8–19 replicate clutches per cross of inbred lines, where each clutch was the product of an independent male × female mating (see Table 2, third column). We fed the freshwater alga *Rhodomonas minuta* to all the copepods in the 0 PSU treatment, and saltwater alga *Rhodomonas salina* to copepods in the 15 PSU treatment, and a 1:1 mixture of *R. minuta* and *R. salina* to the 2.5 PSU treatment. To avoid bacterial infection, we treated all copepods with antibiotic Primaxin® (20 mg/L) every 3–4 days.

STATISTICAL ANALYSIS

To make comparisons among survival of different types of F1 offspring, we analyzed survival data by employing a mixed-effect logistic regression model using the *lme4* package of the statistical software R (Bates et al. 2013, R Core Team 2013). We estimated survival probabilities for the 16 diallel matings (*cross*) under three salinity treatments (0, 2.5, and 15 PSU). Fixed effects included *cross* (genotype effect, 16 levels), *salinity* (three levels), and *cross-by-salinity* interactions (48 levels), whereas random effects included *block* (time when experiment was conducted) and *clutch* (the effect of belonging to the same egg sac). We treated the response variable (*survival from hatching to adult*) as binary. The experiment was carried out in two blocks, with 62 clutches in block 1 and 122 clutches in block 2 (184 clutches in total).

In the mixed-effect logistic regression model, each survival probability takes the form

$$\frac{1}{1+e^{-(\eta_{ij}+b_k+c_m)}}, \tag{1}$$

where the fixed effect η_{ij} is the expected log odds of survival for the cross i at salinity j, b_k is the random effect for *block*, and c_m is the random effect for *clutch*. Distribution of random effects for *block* (b_k) and *clutch* (c_m) are assumed to be normal with mean 0

Table 2. Differences in mean survival (from hatching to adult) at three salinities (0, 2.5, 15 PSU) with standard errors (obtained by 500 parametric bootstrap resamples) for each of 16 matings.

Type of F1 offspring	Cross	No. of replicates (no. of clutches)	Differences in survival probability ± SE		
			0 vs. 2.5 PSU	0 vs. 15 PSU	2.5 vs. 15 PSU
(a) Parental inbred lines	FW_1	14	0.153 ± 0.091	**0.313 ± 0.096***	**0.160 ± 0.071***
	FW_2	16	**0.231 ± 0.100***	**0.606 ± 0.105***	**0.554 ± 0.099***
	SW_1	19	**-0.154 ± 0.066***	**-0.529 ± 0.097***	**-0.375 ± 0.079***
	SW_2	11	**-0.189 ± 0.090***	**-0.550 ± 0.123***	**-0.361 ± 0.110***
(b) Within-salinity F1 crosses	$FW_1 \times FW_2$	10	0.108 ± 0.106	**0.662 ± 0.092***	**0.554 ± 0.111***
	$FW_2 \times FW_1$	8	**0.275 ± 0.128***	**0.607 ± 0.111***	**0.332 ± 0.129***
	$SW_1 \times SW_2$	12	**-0.548 ± 0.103***	**-0.741 ± 0.085***	-0.193 ± 0.106
	$SW_2 \times SW_1$	9	**-0.443 ± 0.114***	**-0.571 ± 0.105***	-0.128 ± 0.123
(c) Between-salinity F1 crosses	$FW_1 \times SW_1$	12	0.039 ± 0.087	-0.003 ± 0.080	-0.042 ± 0.085
	$SW_1 \times FW_1$	11	0.004 ± 0.078	0.071 ± 0.089	0.067 ± 0.089
	$FW_1 \times SW_2$	10	-0.008 ± 0.068	0.032 ± 0.074	0.040 ± 0.074
	$SW_2 \times FW_1$	8	0.080 ± 0.098	0.062 ± 0.093	-0.018 ± 0.103
	$FW_2 \times SW_1$	11	-0.064 ± 0.086	-0.010 ± 0.094	0.054 ± 0.087
	$SW_1 \times FW_2$	12	-0.052 ± 0.095	-0.106 ± 0.091	-0.054 ± 0.090
	$FW_2 \times SW_2$	10	0.034 ± 0.079	0.115 ± 0.084	0.081 ± 0.089
	$SW_2 \times FW_2$	11	-0.016 ± 0.090	-0.022 ± 0.085	-0.006 ± 0.080

*Significant differences ($P < 0.05$) are in bold.

and variance σ^2_{block} and σ^2_{clutch}, respectively ($b_k \sim N(0, \sigma^2_{block})$ for $k = 1, 2$ and $c_m \sim N(0, \sigma^2_{clutch})$ for $m = 1, \ldots, 184$). The estimated mean survival probability for the cross i under salinity j is

$$\hat{\pi}_{ij} = \frac{1}{1 + e^{-\hat{\eta}_{ij}}}, \quad (2)$$

where $\hat{\eta}_{ij}$ is estimated from the model and data.

To make statistical inferences about differences in survival between the F1 crosses (Fig. 2B) and to estimate standard errors for survival probabilities of individual crosses, we implemented parametric bootstrapping (Efron and Tibshirani 1993) with 500 bootstrap replicates. We used the bootstrap approach because formulas for standard errors in mixed-effects logistic regression models are unavailable. To test for differences in survival between crosses we implemented 95% confidence intervals. Specifically, 95% confidence intervals for the difference between means that do not contain 0 indicate a significant difference between two crosses at the 0.05 level ($P < 0.05$).

Results

BENEFICIAL REVERSAL OF DOMINANCE BETWEEN SALINITIES

Our results strongly supported the presence of beneficial reversal of dominance in salinity tolerance between saltwater and freshwater conditions in *E. affinis*. That is, saltwater tolerance was completely dominant under saltwater conditions, whereas freshwater tolerance was completely dominant under freshwater conditions. This reversal of dominance was supported by the lack of significant differences when comparing survival of the between-salinity F1 crosses (Fig. 2B-(c), SW × FW-F1) with that of the within-saltwater F1 crosses (Fig. 2B-(b), SW-F1) under saltwater conditions (Figs. 3 and 4A; Table 4) and with that of the within-freshwater F1 crosses (Fig. 2B-(b), FW-F1) under freshwater conditions (Figs. 3 and 4B, Table 3). Furthermore, the between-salinity F1 crosses (SW × FW-F1) showed evidence of higher survival (overdominance) under conditions suboptimal, that is, at 2.5 PSU, to both saltwater (SW-F1) and freshwater (FW-F1) within-salinity F1 crosses (Table S3), indicating that dominance always shifted in a manner that increased the survival of heterozygotes.

Because we observed the same pattern of dominance in salinity tolerance shifting between saltwater and freshwater conditions in all eight SW × FW-F1 reciprocal crosses (Tables 3 and 4, Figs. 3 and 4), our results provided strong evidence for the complete beneficial reversal of dominance in salinity tolerance. Under saltwater conditions, survival of all eight reciprocal SW × FW-F1 crosses was not significantly different from survival of the within-saltwater F1 crosses (SW-F1), but was significantly higher ($P < 0.05$) than survival of the within-freshwater F1 crosses (FW-F1) (Fig. 4A, Table 4). Likewise, under freshwater conditions survival of all eight SW × FW-F1 crosses was not significantly different from that of the within-freshwater F1 crosses (FW-F1), but was significantly higher ($P < 0.05$) than survival of the within-saltwater F1 crosses (SW-F1) (Fig. 4B, Table 3).

Table 3. Differences in maximum-likelihood (ML) estimates of survival (from hatching to adult) in four inbred lines and their reciprocal crosses under freshwater (0 PSU) conditions.

Cross	FW_1	FW_2	SW_1	SW_2	FW_1xFW_2	FW_2xFW_1	SW_1xSW_2	SW_2xSW_1	FW_1xSW_1	SW_1xFW_1	FW_1xSW_2	SW_2xFW_1	FW_2xSW_1	SW_1xFW_2	FW_2xSW_2	SW_2xFW_2
FW_1	**0.336**	−0.283 (0.129)	0.336 (0.102)	0.336 (0.102)	−0.490 (0.117)	−0.452 (0.130)	0.294 (0.104)	0.252 (0.108)	−0.449 (0.115)	−0.510 (0.107)	−0.547 (0.109)	−0.524 (0.116)	−0.341 (0.127)	−0.373 (0.121)	−0.528 (0.111)	−0.510 (0.114)
FW_2		**0.619**	0.619 (0.121)	0.619 (0.108)	−0.207 (0.121)	−0.169 (0.137)	0.577 (0.109)	0.535 (0.112)	−0.166 (0.123)	−0.227 (0.112)	−0.264 (0.117)	−0.241 (0.122)	−0.058 (0.127)	−0.090 (0.127)	−0.245 (0.120)	−0.227 (0.123)
SW_1			**0.000**	0.000 (0.000)	−0.826 (0.084)	−0.788 (0.104)	−0.042 (0.031)	−0.084 (0.061)	−0.785 (0.083)	−0.846 (0.076)	−0.883 (0.065)	−0.860 (0.076)	−0.677 (0.104)	−0.709 (0.098)	−0.864 (0.071)	−0.846 (0.082)
SW_2				**0.000**	−0.826 (0.084)	−0.788 (0.104)	−0.042 (0.031)	−0.084 (0.082)	−0.785 (0.083)	−0.846 (0.076)	−0.883 (0.065)	−0.860 (0.076)	−0.677 (0.104)	−0.709 (0.098)	−0.864 (0.071)	−0.846 (0.061)
FW_1xFW_2					**0.826**	0.038 (0.123)	0.784 (0.084)	0.742 (0.093)	0.041 (0.106)	−0.020 (0.100)	−0.057 (0.099)	−0.034 (0.107)	0.149 (0.119)	0.117 (0.120)	−0.038 (0.101)	−0.020 (0.106)
FW_2xFW_1						**0.788**	0.746 (0.105)	0.704 (0.111)	0.003 (0.122)	−0.058 (0.119)	−0.095 (0.112)	−0.072 (0.123)	0.111 (0.136)	0.079 (0.127)	−0.076 (0.121)	−0.058 (0.128)
SW_1xSW_2							**0.042**	−0.042 (0.064)	−0.743 (0.085)	−0.804 (0.080)	−0.841 (0.070)	−0.818 (0.079)	−0.635 (0.102)	−0.667 (0.100)	−0.822 (0.076)	−0.804 (0.086)
SW_2xSW_1								**0.084**	−0.701 (0.094)	−0.762 (0.089)	−0.799 (0.084)	−0.776 (0.088)	−0.593 (0.109)	−0.625 (0.103)	−0.780 (0.086)	−0.762 (0.092)
FW_1xSW_1									**0.785**	−0.061 (0.100)	−0.098 (0.096)	−0.075 (0.103)	0.108 (0.111)	0.076 (0.110)	−0.079 (0.100)	−0.061 (0.102)
SW_1xFW_1										**0.846**	−0.037 (0.089)	−0.014 (0.097)	0.169 (0.113)	0.137 (0.111)	−0.018 (0.093)	0.000 (0.102)
FW_1xSW_2											**0.883**	0.023 (0.094)	0.206 (0.113)	0.174 (0.106)	0.019 (0.094)	0.037 (0.097)
SW_2xFW_1												**0.860**	0.183 (0.114)	0.151 (0.111)	−0.004 (0.097)	0.014 (0.109)
FW_2xSW_1													**0.677**	−0.032 (0.122)	−0.187 (0.116)	−0.169 (0.118)
SW_1xFW_2														**0.709**	−0.155 (0.109)	−0.137 (0.112)
FW_2xSW_2															**0.864**	0.018 (0.100)
SW_2xFW_2																**0.846**

Significant ($P < 0.05$) Not significant

Values in cells show pairwise differences in survival between different crosses (row − column values). Bold numbers on the diagonal are ML estimates of survival probabilities for each cross at 0 PSU. Differences in ML estimates of survival probabilities were tested by constructing 95% confidence intervals for mean differences using standard errors (in parentheses) obtained by 500 parametric bootstrap resamples.

These replicated results provided robust support for the complete dominance of saltwater tolerance under saltwater conditions (Figs. 3 and 4A, Table 4) and the complete dominance of freshwater tolerance under freshwater conditions (Figs. 3 and 4B, Table 3).

The saltwater inbred lines (Fig. 3a, SW_1 and SW_2) and their within-salinity F1 crosses (Fig. 3b, SW-F1) exhibited reaction norms of opposite slope across salinities compared to their freshwater counterparts (Fig. 3a, FW_1 and FW_2, and Fig. 3b, FW-F1). The opposing slopes were supported by a significant cross by salinity interaction ($\chi^2_{14} = 320.3, P < 0.001$). Consistent with this significant interaction, the two freshwater parental inbred lines (FW_1 and FW_2) and their reciprocal crosses (FW-F1, i.e., $FW_1 \times FW_2$ and $FW_2 \times FW_1$) displayed significantly higher survival at their native salinity (0 PSU) than at 15 PSU (Fig. 3, blue lines, $P < 0.05$, Table 2), whereas the saltwater inbred lines (SW_1 and SW_2) and their reciprocal crosses (SW-F1, i.e., $SW_1 \times SW_2$ and $SW_2 \times SW_1$) showed the opposite pattern, of significantly lower survival at 0 PSU than at their native 15 PSU (Fig. 3, red lines, $P < 0.05$, Table 2). In sharp contrast, the between-salinity SW × FW-F1 crosses exhibited high survival across all three salinities (Fig. 3c, purple lines, Table 2), showing much flatter reaction norms and no significant cross by salinity interaction ($\chi^2_{14} = 5.399, P = 0.979$). These patterns of survival across salinities supported the presence of beneficial reversal of dominance.

We were able to assess the increase in survival due to heterosis by comparing the survival of the parental inbred lines (Figs. 2B-(a), 3a) with that of the within-salinity crosses (Figs. 2B-(b), 3b, SW-F1 and FW-F1). We did find evidence of heterosis in some instances, where crosses between independently derived lines from a population (SW-F1 and FW-F1) showed higher survival than that of some of the parental inbred lines (Fig. 4). Under freshwater conditions the within-freshwater F1 crosses (FW-F1) showed significantly higher survival relative to the FW_1 parental inbred line only ($P < 0.05$, Fig. 4B; Table 3, difference in survival = 0.49 and 0.45), indicating the presence of heterosis in

Table 4. Differences in maximum-likelihood (ML) estimates of survival (from hatching to adult) in four inbred lines and their reciprocal crosses under saltwater (15 PSU) conditions.

Cross	FW_1	FW_2	SW_1	SW_2	FW_1xFW_2	FW_2xFW_1	SW_1xSW_2	SW_2xSW_1	FW_1xSW_1	SW_1xFW_1	FW_1xSW_2	SW_2xFW_1	FW_2xSW_1	SW_1xFW_2	FW_2xSW_2	SW_2xFW_2
FW_1	**0.023**	0.01 (0.029)	−0.506 (0.100)	−0.527 (0.117)	−0.141 (0.085)	−0.158 (0.105)	−0.76 (0.091)	−0.632 (0.117)	−0.765 (0.079)	−0.752 (0.092)	−0.828 (0.072)	−0.775 (0.097)	−0.664 (0.100)	−0.792 (0.084)	−0.726 (0.096)	−0.846 (0.072)
FW_2		**0.013**	−0.516 (0.101)	−0.537 (0.117)	−0.151 (0.084)	−0.168 (0.104)	−0.77 (0.091)	−0.642 (0.114)	−0.775 (0.079)	−0.762 (0.092)	−0.838 (0.072)	−0.785 (0.097)	−0.674 (0.099)	−0.802 (0.083)	−0.736 (0.098)	−0.856 (0.071)
SW_1			**0.529**	−0.021 (0.131)	0.365 (0.111)	0.348 (0.130)	−0.254 (0.121)	−0.126 (0.134)	−0.259 (0.109)	−0.246 (0.116)	−0.322 (0.111)	−0.269 (0.124)	−0.158 (0.121)	−0.286 (0.113)	−0.22 (0.126)	−0.34 (0.109)
SW_2				**0.55**	0.386 (0.123)	0.369 (0.141)	−0.233 (0.133)	−0.105 (0.150)	−0.238 (0.120)	−0.225 (0.133)	−0.301 (0.132)	−0.248 (0.141)	−0.137 (0.136)	−0.265 (0.132)	−0.199 (0.127)	−0.319 (0.123)
FW_1xFW_2					**0.164**	−0.017 (0.121)	−0.619 (0.102)	−0.491 (0.130)	−0.624 (0.101)	−0.611 (0.112)	−0.687 (0.098)	−0.634 (0.123)	−0.523 (0.116)	−0.651 (0.105)	−0.585 (0.114)	−0.705 (0.095)
FW_2xFW_1						**0.181**	−0.602 (0.126)	−0.474 (0.139)	−0.607 (0.119)	−0.594 (0.127)	−0.67 (0.110)	−0.617 (0.135)	−0.506 (0.134)	−0.634 (0.122)	−0.568 (0.134)	−0.688 (0.116)
SW_1xSW_2							**0.783**	0.128 (0.121)	−0.005 (0.108)	0.008 (0.118	−0.068 (0.103)	−0.015 (0.126)	0.096 (0.116)	−0.032 (0.108)	0.034 (0.117)	−0.086 (0.107)
SW_2xSW_1								**0.655**	−0.133 (0.123)	−0.12 (0.126)	−0.196 (0.121)	−0.143 (0.139)	−0.032 (0.141)	−0.16 (0.128)	−0.094 (0.134)	−0.214 (0.121)
FW_1xSW_1									**0.788**	0.013 (0.106)	−0.063 (0.093)	−0.01 (0.116)	0.101 (0.112)	−0.027 (0.099)	0.039 (0.112)	−0.081 (0.090)
SW_1xFW_1										**0.775**	−0.076 (0.103)	−0.023 (0.125)	0.088 (0.119)	−0.04 (0.106)	0.026 (0.117)	−0.094 (0.107)
FW_1xSW_2											**0.851**	0.053 (0.106)	0.164 (0.107)	0.036 (0.093)	0.102 (0105)	−0.018 (0.091)
SW_2xFW_1												**0.798**	0.111 (0.128)	−0.017 (0.118)	0.049 (0.124)	−0.071 (0.111)
FW_2xSW_1													**0.687**	−0.128 (0.114)	−0.062 (0.127)	−0.182 (0.114)
SW_1xFW_2														**0.815**	0.066 (0.111)	−0.054 (0.100)
FW_2xSW_2															**0.749**	−0.12 (0.114)
SW_2xFW_2																**0.869**

Significant ($P < 0.05$) Not significant

Values in cells show pairwise differences in survival between different crosses (row − column values). Bold numbers on the diagonal are ML estimates of survival probabilities for each cross at 15 PSU. Differences in ML estimates of survival probabilities were tested by constructing 95% confidence intervals for mean differences using standard errors (in parentheses) obtained by 500 parametric bootstrap resamples.

that particular case. Similarly, under saltwater conditions the within-saltwater F1 crosses (SW-F1) showed significantly higher survival relative to the SW_1 parental inbred line ($P < 0.05$, Fig. 4A; Table 4, difference in survival = 0.254), indicative of heterosis.

We found that heterosis could not fully explain the increased survival in the between-salinity crosses (SW × FW-F1) relative to the parental inbred lines (Fig. 4, Tables 3 and 4). As the within-salinity crosses accounted for heterosis resulting from crossing independently derived inbred lines, the increase in survival relative to the within-salinity crosses was most likely due to the effects of dominance at loci affecting salinity tolerance. Higher survival of the between-salinity F1 crosses (SW × FW-F1) relative to that of within-saltwater F1 crosses (SW-F1) under freshwater conditions (Fig. 4B, Table 3) and relative to that of within-freshwater F1 crosses (FW-F1) under saltwater conditions (Fig. 4A, Table 4) revealed the effects of reversal of dominance. This elevated survival of the between-salinity F1 crosses (SW × FW-F1) beyond that of the within-salinity F1 crosses (SW-F1 or FW-F1) under maladaptive conditions revealed the effects of dominance of the beneficial alleles (Figs. 3 and 4, purple lines or bars).

When we compared survival at an intermediate salinity (2.5 PSU) that was suboptimal for both the saline and freshwater populations, we found evidence of overdominance in the between-salinity F1 crosses (SW × FW-F1) relative to the within-salinity F1 crosses (SW-F1 and FW-F1) (Fig. 3; Table S3). At this intermediate salinity (2.5 PSU), several between-salinity F1 crosses ($SW_1 \times FW_1$, $FW_1 \times SW_2$, $FW_2 \times SW_2$, and $SW_2 \times FW_2$) displayed significantly higher survival relative to that of the freshwater $FW_2 \times FW_1$-F1 cross and both saline SW-F1 crosses ($P < 0.05$, Table S3), indicating overdominance (heterozygote advantage). The other four SW × FW-F1 crosses exhibited survival that was not significantly different from that of the SW-F1 and FW-F1 crosses ($P > 0.05$). Thus, overall dominance shifted across salinities in a manner that always optimized survival of

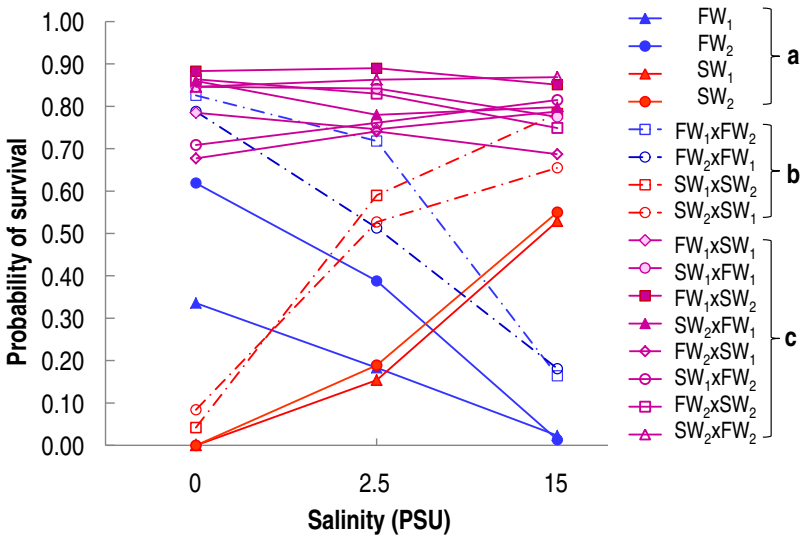

Figure 3. Maximum-likelihood estimates of probabilities of survival from hatching to adult for (a) two freshwater and two saltwater parental inbred lines, (b) reciprocal within-salinity F1 crosses, and (c) reciprocal between-salinity F1 crosses. Survival of the between-salinity F1 crosses (c, purple lines) was not significantly different from survival of freshwater F1 crosses (b, blue dashed lines) under freshwater conditions or survival of saltwater F1 crosses (b, red dashed lines) under saltwater conditions. This pattern of survival strongly supported the presence of beneficial reversal of dominance (also see Tables 3 and 4).

the between-salinity (SW × FW-F1) crosses, including at the intermediate salinity.

MARGINAL OVERDOMINANCE ACROSS SALINITIES

Although the presence of complete reversal of dominance between the saltwater and freshwater environments would consequently result in marginal overdominance, we also formally tested for marginal overdominance (specifically, arithmetic mean overdominance) in salinity tolerance across all three salinities. The presence of marginal overdominance in salinity tolerance was evident from the higher marginal survival (mean survival across all three salinities) of the between-salinity F1 crosses (SW × FW-F1) relative to the within-salinity F1 crosses (SW-F1 and FW-F1) (Table 5, Fig. 5). We found significantly higher marginal survival in seven of eight between-salinity crosses (SW × FW-F1) relative to the within-salinity crosses (SW-F1 and FW-F1) (Table 5, $P < 0.05$, based on 95% confidence intervals obtained by 500 bootstrap replicates). In the only case where survival of the between-salinity cross was not significantly higher than survival of the within-salinity F1 crosses, the between-salinity cross ($FW_2 \times SW_1$) showed an average survival probability across salinities of 0.70 ± 0.07 (Table S2), which was higher than marginal survivals of the $FW_1 \times FW_2$ (0.55 ± 0.09) and $FW_2 \times FW_1$ (0.50 ± 0.1) crosses (Tables 5 and S2). Overall, these results provided strong evidence for marginal overdominance in Wallace's sense (1968, where marginal overdominance = arithmetic mean overdominance) in salinity tolerance in *E. affinis*.

Discussion

EVIDENCE OF REVERSAL OF DOMINANCE AND MARGINAL OVERDOMINANCE IN SALINITY TOLERANCE

For a locus with pleiotropic effects, dominance relationships between two alleles can vary across traits. Beneficial reversal of dominance is a specific case of such variation in dominance, where an allele that is beneficial for some traits and detrimental for other traits is always dominant in the traits for which it is beneficial and recessive in the traits for which it is detrimental (Kidwell et al. 1977; Gillespie 1978; Hoekstra et al. 1985; Curtsinger et al. 1994; Fry 2010). Relevant traits can include fitness (or components of fitness) in different environments. To provide evidence for beneficial reversal of dominance, we demonstrated that survival of the between-salinity F1 crosses (SW × FW-F1, carrying both freshwater and saltwater tolerance alleles) was not significantly different from that of the freshwater crosses (FW-F1, carrying only freshwater tolerance alleles) under freshwater conditions (Table 3, Figs. 3 and 4B) and not significantly different from that of the saltwater crosses (SW-F1, carrying only saltwater tolerance alleles) under saltwater conditions (Table 4, Figs. 3 and 4A). Additionally, we showed that survival of the between-salinity F1 crosses (SW × FW-F1) was significantly higher ($P < 0.05$) than that of the freshwater crosses (FW-F1) under saltwater conditions (Table 3, Figs. 3 and 4A) and also higher than that of the saltwater crosses (SW-F1) under freshwater conditions (Table 4, Figs. 3 and 4B) such that the heterozygote exhibited the phenotype of the more-fit allele in each environment. These results together

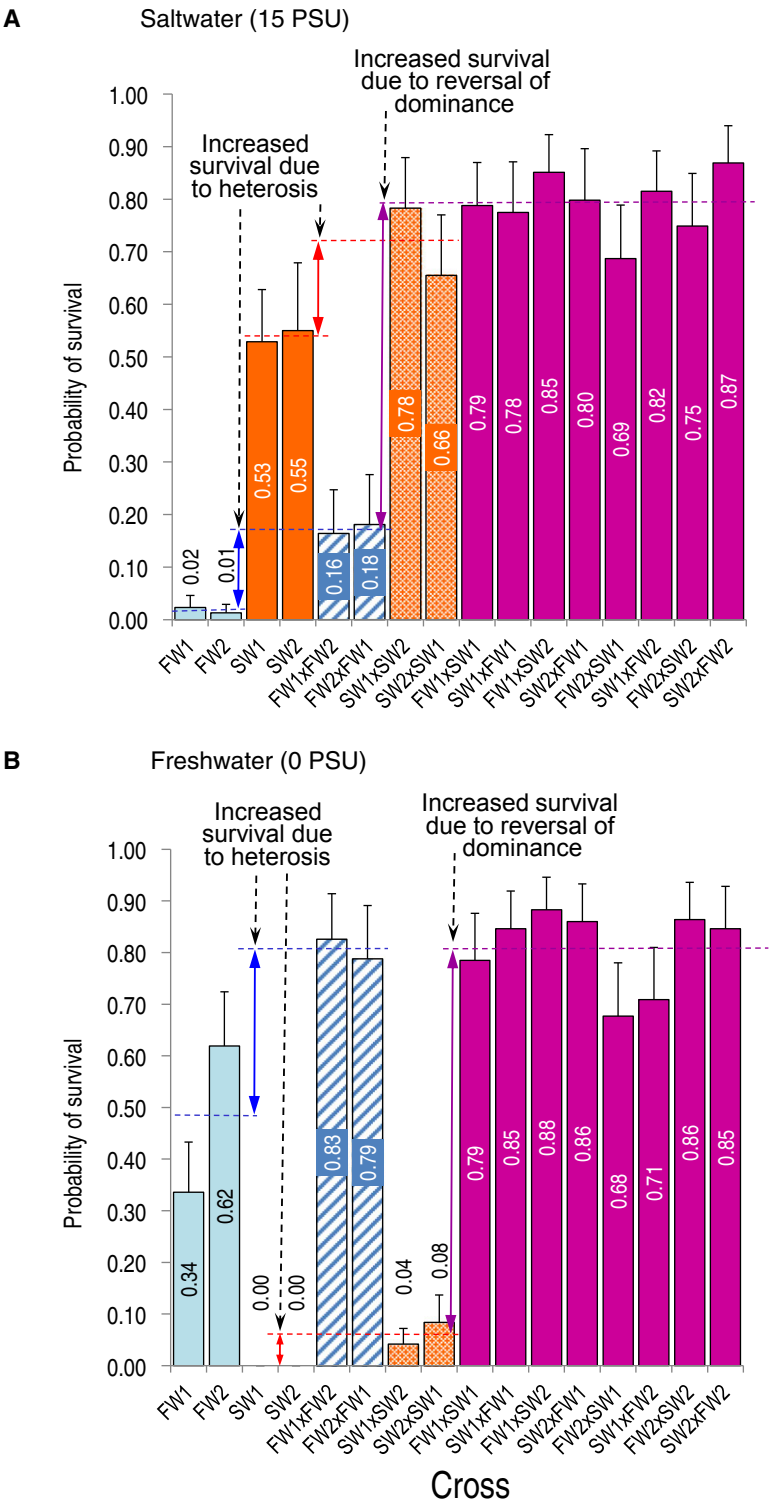

Figure 4. Survival from hatching to adult (maximum-likelihood estimates, numbers in or above the bars) in four inbred lines and their reciprocal crosses under (A) saltwater conditions (15 PSU) and (B) freshwater conditions (0 PSU). Red arrows (middle) indicate the increase in survival due to heterosis alone in the saltwater within-salinity crosses (SW-F1, light red bars), calculated as the difference between mean survival of saltwater within-salinity crosses (SW-F1) and saltwater parental inbred lines (SW_1 and SW_2, red bars). Blue arrows (left) indicate the increase in survival due to heterosis in the freshwater within-salinity crosses (FW-F1, blue striped bars), calculated as the difference between mean survival of FW-F1 and freshwater parental inbred lines (FW_1 and FW_2, blue bars). Purple arrows (right) indicate the increase in survival in the between-salinity F1 crosses (SW × FW-F1, purple bars) due to reversal of dominance, calculated as the difference between mean survival of the between-salinity F1 crosses (SW × FW-F1) and the within-salinity F1 crosses (SW-F1 and FW-F1).

Table 5. Differences in maximum-likelihood (ML) estimates of mean survival (from hatching to adult) across three salinities (marginal survival) in four parental inbred lines and their reciprocal crosses.

Cross	FW_1	FW_2	SW_1	SW_2	FW_1xFW_2	FW_2xFW_1	SW_1xSW_2	SW_2xSW_1	FW_1xSW_1	SW_1xFW_1	FW_1xSW_2	SW_2xFW_1	FW_2xSW_1	SW_1xFW_2	FW_2xSW_2	SW_2xFW_2
FW_1	**0.181**	−0.155 (0.073)	−0.028 (0.059)	−0.040 (0.068)	−0.371 (0.089)	−0.317 (0.098)	−0.245 (0.083)	−0.242 (0.087)	−0.593 (0.067)	−0.633 (0.067)	−0.684 (0.059)	−0.631 (0.069	−0.519 (0.074)	−0.581 (0.069)	−0.629 (0.067)	−0.671 (0.062)
FW_2		**0.336**	0.126 (0.073)	0.114 (0.081)	−0.216 (0.098)	−0.162 (0.107)	−0.091 (0.091)	−0.087 (0.095)	−0.439 (0.079)	−0.478 (0.079)	−0.530 (0.077)	−0.476 (0.082)	−0.364 (0.084)	−0.426 (0.082)	−0.474 (0.079)	−0.517 (0.079)
SW_1			**0.209**	−0.012 (0.065)	−0.343 (0.088)	−0.289 (0.096)	−0.217 (0.078)	−0.214 (0.083)	−0.565 (0.064)	−0.604 (0.062)	−0.656 (0.061)	−0.602 (0.069)	−0.490 (0.072)	−0.552 (0.067)	−0.600 (0.063)	−0.643 (0.060)
SW_2				**0.221**	−0.330 (0.096)	−0.277 (0.104)	−0.205 (0.087)	−0.201 (0.091)	−0.553 (0.075)	−0.592 (0.073)	−0.644 (0.068)	−0.590 (0.075)	−0.478 (0.082)	−0.540 (0.076)	−0.588 (0.074)	−0.631 (0.071)
FW_1xFW_2					**0.552**	0.054 (0.115)	0.125 (0.101)	0.129 (0.109)	−0.223 (0.093)	−0.262 (0.092)	−0.313 (0.090)	−0.260 (0.094)	−0.148 (0.097)	−0.210 (0.097)	−0.258 (0.094)	−0.301 (0.092)
FW_2xFW_1						**0.498**	0.072 (0.110)	0.075 (0.111)	−0.276 (0.102)	−0.316 (0.100)	−0.367 (0.099)	−0.314 (0.104)	−0.202 (0.105)	−0.264 (0.103)	−0.312 (0.098)	−0.354 (0.103)
SW_1xSW_2							**0.426**	0.004 (0.100)	−0.348 (0.088)	−0.387 (0.083)	−0.439 (0.082)	−0.385 (0.089)	−0.273 (0.091)	−0.335 (0.088)	−0.383 (0.082)	−0.426 (0.080)
SW_2xSW_1								**0.423**	−0.352 (0.093)	−0.391 (0.093)	−0.442 (0.088)	−0.389 (0.092)	−0.277 (0.096)	−0.339 (0.090)	−0.387 (0.090)	−0.429 (0.090)
FW_1xSW_1									**0.774**	−0.039 (0.072)	−0.091 (0.068)	−0.037 (0.073)	0.075 (0.078)	0.013 (0.073)	−0.035 (0.070)	−0.078 (0.067)
SW_1xFW_1										**0.814**	−0.010 (0.067)	0.002 (0.074)	0.114 (0.077)	0.052 (0.071)	0.004 (0.067)	−0.039 (0.068)
FW_1xSW_2											**0.865**	0.053 (0.069)	0.166 (0.076)	0.104 (0.068)	0.056 (0.065)	0.013 (0.062)
SW_2xFW_1												**0.812**	0.112 (0.083)	0.050 (0.074)	0.002 (0.073)	−0.041 (0.071)
FW_2xSW_1													**0.700**	−0.062 (0.082)	−0.110 (0.080)	−0.153 (0.079)
SW_1xFW_2														**0.762**	−0.048 (0.072)	−0.091 (0.070)
FW_2xSW_2															**0.810**	−0.043 (0.067)
SW_2xFW_2																**0.852**

Significant ($P < 0.05$) Not significant

Values in cells show pairwise differences in mean survival between different crosses (row − column values). Bold numbers on the diagonal are ML estimates of survival probabilities for each cross. Differences in ML estimates of survival probabilities were tested by constructing 95% confidence intervals for mean differences using standard errors (in parentheses) obtained by 500 parametric bootstrap resamples.

provided support for the complete dominance of freshwater tolerance under freshwater conditions and the complete dominance of saltwater tolerance under saltwater conditions.

Our results indicated that higher survival of the between-salinity (SW × FW-F1) crosses, relative to that of the within-salinity crosses at their less-favored salinities (Fig. 4), was not simply a consequence of heterosis. We accounted for the effects of heterosis by comparing survival of the between-salinity crosses to that of the within-salinity crosses (SW-F1 and FW-F1), which were performed between inbred lines that were independently derived from the ancestral (wild saline or wild freshwater) populations. F1 offspring from the within-salinity crosses reveal the degree of heterosis arising purely from crossing two different inbred lines (each potentially suffering from some degree of inbreeding depression). These within-salinity crosses did in some instances show higher survival, indicative of heterosis, relative to the parental inbred lines (Fig. 4A and B, Tables 3 and 4, see Results). As the within-salinity crosses accounted for the effects of heterosis, the higher survival of the between-salinity F1 crosses beyond that of the within-salinity F1 crosses under maladaptive conditions was likely the result of dominance of the conditionally beneficial allele (i.e., due to beneficial reversal of dominance), and not the result of simple heterosis.

We also formally estimated the effect of reversal in dominance on marginal survival (the mean survival across all three environments, i.e., 0, 2.5, and 15 PSU). We found significantly higher marginal survival of the between-salinity SW × FW-F1 crosses relative to both within-salinity F1 crosses (SW-F1 and FW-F1) (Table 5, Fig. 5). This finding directly provides evidence for marginal overdominance in Wallace's (1968) sense of arithmetic mean overdominance (Fig. 5, Table 5) and presents a model for the protection of polymorphism in a population (see Hoekstra et al. 1985).

In addition to complete reversal of dominance between saltwater and freshwater conditions, we also observed overdominance at the intermediate salinity (2.5 PSU), which was suboptimal

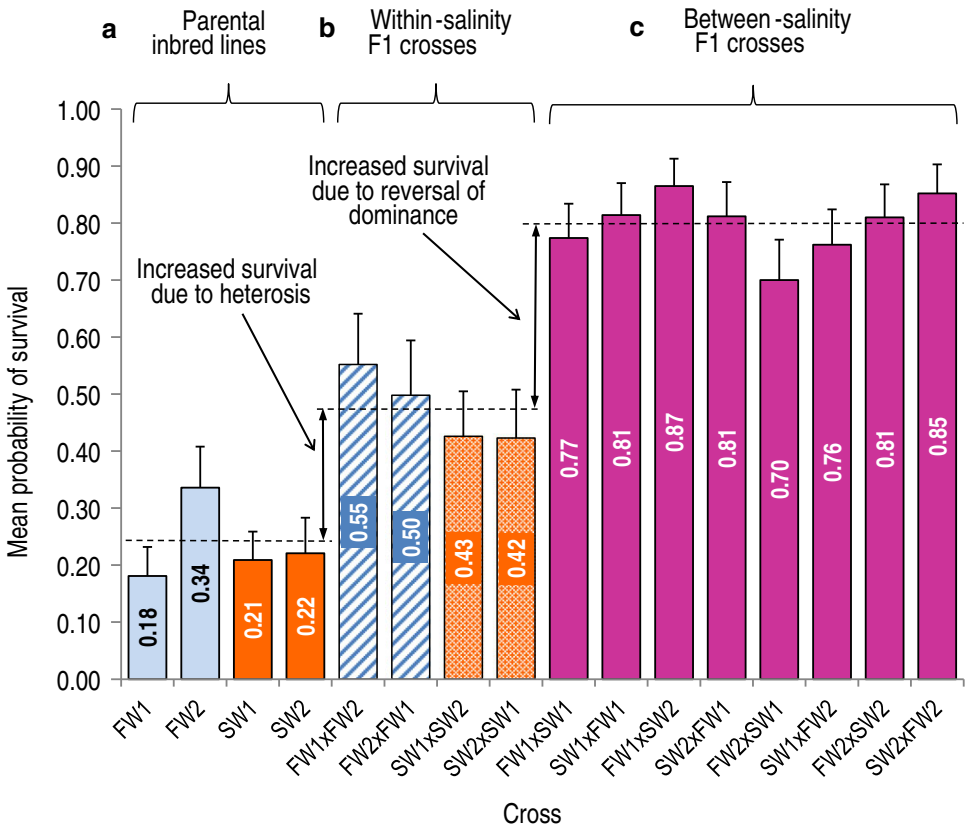

Figure 5. **Marginal survival from hatching to adulthood (maximum-likelihood estimates, numbers in the bars) across all three salinities (0, 2.5, and 15 PSU) in parental inbred lines and their reciprocal crosses. Standard error estimates were obtained by 500 parametric bootstrap resamples (see Methods). The crosses shown are (a) parental inbred lines, (b) within-salinity F1 crosses, between inbred lines independently derived from a population, and (c) between-salinity F1 crosses (SW × FW-F1). Differences in survival between the parental inbred lines (a) and the within-salinity F1 crosses (b) were due to heterosis. Differences in survival between the between-salinity F1 crosses (c) and the within-salinity F1 crosses (b) were due to beneficial reversal of dominance in salinity tolerance. Overall higher survival of the between salinity SW × FW-F1 crosses (c) relative to the within-salinity F1 crosses (b) ($P < 0.05$) provided evidence for marginal overdominance in salinity tolerance.**

for both freshwater and saltwater parental inbred lines. Overdominance at the intermediate salinity was indicated by significantly higher survival of four of the between-salinity (SW × FW-F1) crosses relative to the within-salinity F1 crosses (SW-F1 and FW-F1) (Table S2, Fig. 3). Thus, overdominance at the intermediate salinity along with the reversal of dominance between saline and freshwater conditions acted to optimize survival of the between-salinity crosses (SW × FW-F1) across salinities.

Beneficial reversal of dominance for survival to adulthood (i.e., the fitness-related trait we measured) need not necessarily imply beneficial reversal of dominance for total fitness. However, survival to adulthood is one of the most central components of fitness. In addition, in prior studies we have not observed any trade-offs in survival among life-history stages (Lee et al. 2003, 2007, 2013). Salinity tolerance in *E. affinis* appears to predominantly reflect appropriate osmoregulation—for example, freshwater adapted lines (with high survival in freshwater) exhibit a less-extreme reduction in blood hemolymph concentration at low salinity (i.e., better uptake and conservation of ions) relative to saline-adapted lines (Lee et al. 2012). Lines raised at the salinity to which they are adapted also appear to show more typical behavior (e.g., quicker swimming speed and escape responses) (C. E. Lee, pers. obs.). We expect that, with respect to salinity, fecundity and total fitness would show a positive correlation with survival to adulthood, because all these aforementioned traits would be similarly affected by the ability to osmoregulate appropriately at each salinity. Thus, total fitness is likely to show a similar pattern of beneficial reversal of dominance as survival to adulthood, though this remains to be formally tested.

Our data appear to provide prima facie evidence for complete beneficial reversal of dominance for a core fitness-related

trait. Alternatively, the data could be explained, without invoking reversal of dominance, by complementation of loss-of-function alleles in a multilocus model (Kawecki 1997). Kawecki (1997) has shown that, for a species initially occupying multiple habitats, deleterious mutations at loci that provide crucial habitat-specific functions could drive specialization. Loss-of-function mutations at loci that are nonessential in a given habitat could result in different loci retaining function in different habitats. For example, in a freshwater population, functional alleles could be lost at loci required for survival in saline habitats and vice versa. In such a case, in freshwater × saline F1 offspring, the functional allele at each locus would compensate for the nonfunctional allele. Thus, freshwater × saline F1 crosses could then display marginal overdominance, with high fitness across all salinities, due to simple dominance of functional over nonfunctional alleles at each locus (i.e., without reversal of dominance).

However, such a mechanism as described above is unlikely to explain the data presented here. Specialization through the accumulation of nonfunctional alleles would be a protracted process (driven by mutation pressure), whereas freshwater adaptation has occurred very rapidly and recently in invasive populations of *E. affinis*. More importantly, the negative genetic correlations observed between freshwater and saltwater tolerance in *E. affinis* (Lee et al. 2003, 2007) indicate the presence of antagonistic pleiotropy, with the same loci affecting survival in both environments. Such antagonistic pleiotropy is also consistent with the observation that in freshwater-adapted populations of *E. affinis*, levels of ion-motive V-type ATPase activity are elevated in freshwater-reared animals and concomitantly reduced in saline-reared animals, when compared to saline-adapted populations (i.e., evidence of a genetic trade-off for this crucial ion uptake enzyme) (Lee et al. 2011). Thus, reversal of dominance remains the most plausible explanation for our data.

In general, the extent of dominance is not a fixed value, but can vary as a function of genetic background or environmental factors (Bourguet et al. 1996; Billiard and Castric 2011). Rather than conceptualizing our experiment in terms of dominance at separate traits (where survival at each salinity is viewed as its own trait), our experiment could equivalently be conceptualized as measuring a single trait (survival to adulthood) across a set of environments (range of salinities), with reversal of dominance reflecting plasticity of dominance across environments for the alleles governing the trait. One example of dramatic plastic shifts in dominance across environmental conditions comes from a study of insecticide resistance in the mosquito *Culex pipiens*, conferred by an allele of the *Ace* locus that encodes an acetylcholinesterase that is insensitive to organophosphorus insecticides (Bourguet et al. 1996). Depending on environmental conditions, levels of dominance of insecticide resistance varied from almost complete dominance to almost complete recessivity (Bourguet et al. 1996). However, although this prior study and various others have demonstrated plasticity of dominance across environmental conditions, we are unaware of prior studies with results analogous to ours, where the heterozygote shows fitness (or a major component of fitness, such as survival to adulthood) equal to, alternately, the better of the two homozygotes across the range of an environmental variable.

OTHER EMPIRICAL EXAMPLES OF BENEFICIAL REVERSAL OF DOMINANCE

There are few good, explicit examples of beneficial reversal of dominance in the literature. A study of a locus with antagonistic pleiotropic effects on viability, developmental time, and mating ability in the moth *Ephestia kuhniella* (Caspari 1950) is sometimes cited as an empirical example of reversal of dominance. However, the inferences in this study were somewhat indirect because, for the locus being studied, only one of the two classes of homozygotes could be distinguished from the heterozygote. Thus, as noted by Curtsinger et al. (1994), this study could not distinguish between overdominance and reversal of dominance in all three fitness components (the heterozygote was, for each component, at least equal to and possibly superior to the better homozygote). A cross between inbred lines of *Zea mays* (corn) with different temperature optima for lateral root growth resulted in a hybrid for which lateral root growth at each temperature was comparable to the better parent. (Hund et al. 2012). However, the data were somewhat ambiguous (due to a limited number of replicates), a cross in the reciprocal direction (with the opposite inbred line serving as the male vs. female parent) did not produce the same result, and crosses between other inbred lines did not appear to behave similarly.

A few examples demonstrate apparent beneficial reversal of dominance more clearly. Curtsinger et al. (1994) point out that sickle cell anemia could be conceptualized as a case of beneficial reversal of dominance (and the same interpretation could be applied to other genetic disorders that confer malaria resistance, such as thalassemia) (López et al. 2010). With sickle cell anemia, the wild-type allele shows near-complete dominance with respect to sickle cell disease, such that the heterozygotes generally do not display the disease, whereas the sickle cell allele displays dominance with respect to malaria resistance. Warfarin resistance in rats furnishes another example, where warfarin resistance due to mutation of the VKORC1 gene is associated with an increased dietary requirement for vitamin K, and an associated substantial fitness cost in resistant homozygotes (Kohn et al. 2003). The wild-type allele is dominant for low vitamin K requirement (i.e., heterozygotes, like wild-type homozygotes, only require a low level of dietary vitamin K) and the warfarin-resistance allele is dominant for warfarin resistance. Yet another example is the *csr1–1* chlorsulfuron herbicide resistance allele in *Arabidopsis thaliana*,

which carries a fitness cost in resistant homozygotes. In this case, the *csr1–1* allele is dominant for herbicide resistance and the wild-type allele shows essentially complete dominance with respect to fitness cost (Roux et al. 2004). In these examples, of sickle cell anemia, VKORC1 mutant, and *csr1–1* alleles, homozygotes carrying the mutant allele (i.e., the allele conferring tolerance or resistance) suffer deleterious effects, which are present even in the environment for which the mutant allele is the optimal allele. For example, a sickle cell homozygote in an environment with a high incidence of malaria will have increased malaria resistance, but will also bear the large fitness cost of sickle cell disease.

In contrast to the cases above, for salinity tolerance in the copepod *E. affinis*, the optimal alleles in the homozygous state allow near full survival and high absolute fitness in their optimal environment. Moreover, reversal of dominance appears complete in the heterozygote, with the optimal alleles in each environment showing full dominance. We will note that the warfarin system could hypothetically provide results similar to ours depending on the design of the experiment, as the fitness cost in the warfarin system is not absolute, but depends on an environmental variable. An experimental design using two environments, one with normal food and a second with food supplemented with high levels of vitamin K and warfarin, should theoretically show high absolute fitness of the optimal homozygote and the heterozygote in both environments. However, as it currently stands, our study is the only one that fully empirically demonstrates the complete beneficial reversal of dominance of fitness across environments.

ORIGINS AND EVOLUTION OF REVERSAL OF DOMINANCE

Reversal of dominance across environments might arise automatically due to the better-fit allele in a given environment compensating for the reduced function of the allele maladapted to that environment (i.e., where dominance is a simple consequence of nonlinearities in metabolic or developmental systems; Wright 1934; Kacser and Burns 1981; Gilchrist and Nijhout 2001). Alternatively, it is theoretically possible that plasticity in dominance could be selected for, to produce higher dominance of conditionally beneficial alleles for the conditions under which they are beneficial (Bourguet 1999; Otto and Bourguet 1999; Rice 2002). Such selection for reversal of dominance might be particularly intense in fluctuating environments.

In some cases, the segregation load associated with marginal overdominance could ultimately be resolved by gene duplication. A gene duplication combining two antagonistically selected alleles that exhibit marginal overdominance in a heterozygote could allow fixation of permanent "overdominance" for a gene that was previously polymorphic (Haldane 1932; Spofford 1969; Labbé et al. 2014). However, for many genes, such a duplication might prove detrimental due to disruptions in gene dosage.

EVOLUTIONARY IMPLICATIONS OF BENEFICIAL REVERSAL OF DOMINANCE AND MARGINAL OVERDOMINANCE

Role in maintaining genetic variation

Marginal overdominance in fitness across environments has profound implications for the maintenance of polymorphism and adaptation in temporally varying environments. With marginal overdominance, survival of the heterozygotes increases, such that a population has a higher chance of maintaining genetic variation in the face of changing environments. Here we found arithmetic mean overdominance for a core component of fitness (survival to adulthood). Arithmetic mean overdominance is more stringent than both harmonic mean overdominance (where harmonic mean overdominance for fitness is required to indefinitely maintain protected polymorphism under a basic model of spatial heterogeneity) and geometric mean overdominance (where geometric mean overdominance for fitness is required to indefinitely maintain protected polymorphism under a basic model of temporal fluctuation).

Beneficial reversal of dominance, as found in this study, can greatly strengthen the magnitude of marginal overdominance. With changes in the environment, the less-favored allele would be masked from negative selection in the heterozygous state (i.e., because the heterozygote would be close in fitness to the fitter homozygote in each environment). Under a variety of models, theoretical analyses have found that for antagonistically selected alleles, protected polymorphism can be maintained when conditionally beneficial alleles are dominant in the conditions under which they are beneficial (Kidwell et al. 1977; Curtsinger et al. 1994; Epinet and Lenormand 2009; Fry 2010; Connallon and Clark 2012b). In the case of complete dominance (Table 6, $h_1 = h_2 = 0$), reversal of dominance would satisfy the requirement for arithmetic mean overdominance across environments, that is, marginal overdominance as defined by Wallace (1968). If alleles show partial dominance (Table 6, $0 < [h_1, h_2] < 0.5$), beneficial reversal of dominance could result in geometric mean or harmonic mean overdominance (Levene 1953; Gillespie 1973; Felsenstein 1976; Hoekstra et al. 1985). Conditions for geometric mean or harmonic mean overdominance are less stringent than for arithmetic mean overdominance, because the geometric mean or harmonic mean is always less than or equivalent to the arithmetic mean (Felsenstein 1976). Reversal of dominance and spatiotemporally varying selection could thus act in concert to maintain protected polymorphisms, as the resulting marginal overdominance would assure that the less-favored allele is protected against negative selection during environmental change.

Table 6. The simplest model of antagonistic selection involves one locus with two alleles. Beneficial reversal of dominance occurs when $0 \leq [h_1, h_2] < 0.5$ (Curtsinger et al. 1994). If $h_1 = h_2 = 0$ (complete dominance), beneficial reversal of dominance would result in marginal overdominance in Wallace's sense (1968), where the arithmetic mean of heterozygotes is greater than that of homozygotes (arithmetic mean overdominance; Wills 1975; Felsenstein 1976). Marginal overdominance could also occur when the geometric or harmonic mean fitness of the heterozygote across environments is greater than that of both homozygotes (Levene 1953; Gillespie 1973).

	Genotype		
	A_1A_1	A_1A_2	A_2A_2
Environmental (fitness) context 1	1	$1 - h_1s_1$	$1 - s_1$
Environmental (fitness) context 2	$1 - s_2$	$1 - h_2s_2$	1
Marginal overdominance if:	$\bar{w}_{A_1A_1}$	$< \bar{w}_{A_1A_2} >$	$\bar{w}_{A_1A_2}$

$\bar{w}_{AiAi}$ = arithmetic, geometric, or harmonic mean fitness of genotype A_iA_i; s_i = selection coefficient; h_i = dominance coefficient; $i = 1$ or 2.

Not only would beneficial reversal of dominance increase the strength of balancing selection for protected polymorphisms, but, in the presence of recurrent mutation, it should also greatly increase genetic variance across a far wider parameter range and to a greater extent than could be produced by protected polymorphisms alone (Connallon and Clark 2012b). The importance of the latter phenomenon has often been overlooked. Although there is copious literature on conditions required to indefinitely maintain protected polymorphisms, there has been relatively scant attention paid to the potential net positive effect of antagonistic selection on genetic variance when alleles are not preserved indefinitely. Recent theoretical developments suggest that transient balanced polymorphism may be very common in diploids, with antagonistic selection exhibiting a potentially large genetic variance inflating effect under conditions that would not sustain protected polymorphism (Bürger and Gimelfarb 2002; Kelly 2006; Sellis et al. 2011; Connallon and Clark 2012b; Delph and Kelly 2014).

Reversal of dominance could also have potential implications for evolution of mating systems (Epinet and Lenormand 2009). In a spatially heterogeneous habitat with partial migration between niches, beneficial reversal of dominance may allow maintenance of high levels of locally maladaptive alleles, increasing inbreeding depression (as a reflection of segregation load) upon assortative mating. This may disfavor the evolution of assortative mating and inhibit speciation.

Given the exposure of many organisms to continuously changing environments, marginal overdominance as a mode of balancing selection might be widespread across taxa. For example, balancing selection via marginal overdominance may contribute to the observation that the frequency of *Drosophila melanogaster* polymorphisms at hundreds of loci oscillates reproducibly across seasons (Bergland et al. 2014). Numerous studies suggest theoretical potential for higher genetic variation in organisms that originate from fluctuating environments (Korol et al. 1996; Kondrashov and Yampolsky 1996; Burger and Gimelfarb 2002; Hedrick et al. 2002; Lee and Gelembiuk 2008).

It is likely that the spatiotemporal variation in salinity that *E. affinis* experiences in its native estuarine environment (Winkler et al. 2008) combined with marginal overdominance in salinity tolerance would promote the maintenance of polymorphism at salinity tolerance loci in the wild. In the estuarine habitat, salinity levels are spatially heterogeneous and large fluctuations in salinity occur on both seasonal and shorter timescales. In the case of *E. affinis*, it appears that dominance relationships shift across environmental conditions such that both saltwater and freshwater alleles are protected from removal by natural selection in the heterozygous state. The presence of beneficial reversal of dominance and marginal overdominance that we found in *E. affinis* in this study is concordant with previously reported high levels of genetic variance in salinity tolerance observed in both saltwater and freshwater populations of *E. affinis* (Lee et al. 2003, 2007). For example, recessivity of saltwater tolerance in freshwater could explain the presence of saltwater tolerant alleles in the freshwater habitat despite the constant freshwater conditions that would select against them.

Implications for adaptation during invasions

It is increasingly recognized that, in many instances, biological invasions require adaptation to the new range (Carroll et al. 2001; Lee 2002; Dambrowski and Feder 2007; Prentis et al. 2008; Colautti and Barrett 2013; Sultan et al. 2013; Vandepitte et al. 2014). Populations may successfully invade into "black hole sink" environments, with novel abiotic and biotic conditions, in which they could not persist without evolutionary rescue (Holt et al. 2003; Chevin and Lande 2010). Successful invasions by *E. affinis* (via ballast water discharges and canal building) into bodies of freshwater (with ionic concentrations orders of magnitude lower than the native estuarine range) during the last several decades represent one such example (Lee 1999, 2003). These invasions have been shown to constitute evolutionary events (Lee et al. 2003, 2007, 2011, 2012, 2013).

Marginal overdominance would provide conditions for the maintenance of variation in temporally and spatially varying environments and would lead to elevated levels of standing genetic variation that could facilitate rapid adaptation during invasions into novel habitats. Invasions into novel environments would be facilitated, as successful invasion often requires very rapid

adaptation. Such adaptation is more likely to occur from standing genetic variation rather than from de novo beneficial mutations arising during invasion events (Innan and Kim 2004; Colosimo et al. 2005; Lee et al. 2007; Barrett and Schulter 2008; Prentis et al. 2008). Thus, marginal overdominance in salinity tolerance in *E. affinis* would not only result in higher survival in response to temporally varying environments within the native range, but also in its greater potential for colonization of and adaptation to new environments.

Reversal of dominance might also increase initial rates of survival and reduce the risk of population extinction upon introduction into a new environment (e.g., when a stock of estuarine *E. affinis* is transplanted to a freshwater environment through ballast water discharge). In a species invasion, the inoculum into the new range typically consists of a relatively limited number of individuals. Reversal of dominance would effectively broaden the tolerance range, providing more individuals with sufficient absolute fitness to survive the new environment. Survival of a lineage during the initial generations of an invasion is a precondition for ultimate evolutionary rescue and persistence in a novel environment (Chevin and Lande 2010; Palmer and Feldman 2012). As the favorable allele should be effectively dominant in the new habitat (and thus visible to selection as heterozygotes), reversal of dominance should also accelerate initial adaptation; though, once the favored allele reaches high frequency, complete adaptation should be retarded, as the unfavorable allele would be masked from selection. It appears that an evolutionary history in variable environments might correspond to invasiveness of species given the large number of invasive populations that originates from temporally varying disturbance-prone environments (Lee and Gelembiuk, 2008). Such varying environments might often be crucial for enabling marginal overdominance to maintain polymorphism and high levels of standing genetic variance at key traits that might undergo selection during habitat change. Specifically, adaptation might be facilitated along dimensions corresponding to environmental characteristics that are subject to fluctuating selection in the native environment (e.g., salinity in the case of *E. affinis*).

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Maximum-likelihood estimates of probabilities of survival from hatching to adult (± standard errors obtained from parametric bootstrapping) in four inbred lines and their reciprocal crosses at three different salinities.

Table S2. Maximum-likelihood estimates (ML) of probabilities for marginal survival (mean survival across all three salinities, 0, 2.5, and 15 PSU) from hatching to adult.

Table S3. Differences in maximum-likelihood (ML) estimates of survival (from hatching to adult) in four parental inbred lines and their reciprocal crosses at 2.5 PSU.

Erratum for: “Testing for beneficial reversal of dominance during salinity shifts in the invasive copepod *Eurytemora affinis*, and implications for the maintenance of genetic variation” by Posavi et al., which was published in November 2014 issue of Evolution Vol. 68, No. 11, pp. 3166–3183.

The authors would like to make following correction:

At the bottom of the right-hand column on page 3174 in the section on *EVIDENCE OF REVERSAL OF DOMINANCE AND MARGINAL OVERDOMINANCE IN SALINITY TOLERANCE* the text currently states:

> “Additionally, we showed that survival of the between-salinity F1crosses (SW x FW-F1) was significantly higher ($P < 0.05$) than that of the freshwater crosses (FW-F1) under saltwater conditions (Table 3, Figs. 3 and 4A) and also higher than that of the saltwater crosses (SW-F1) under freshwater conditions (Table 4, Figs. 3 and 4B) such that the heterozygote exhibited the phenotype of the more-fit allele in each environment.”

The text should instead read [*changed text is underlined*]:

> “Additionally, we showed that survival of the between-salinity F1crosses (SW x FW-F1) was significantly higher ($P < 0.05$) than that of the freshwater crosses (FW-F1) under saltwater conditions (Table 4, Figs. 3 and 4A) and also higher than that of the saltwater crosses (SW-F1) under freshwater conditions (Table 3, Figs. 3 and 4B) such that the heterozygote exhibited the phenotype of the more-fit allele in each environment.”

Table S1. Maximum-likelihood estimates of probabilities of survival from hatching to adult (± standard errors obtained from parametric bootstrapping) in four inbred lines and their reciprocal crosses at three different salinities. Statistically significant differences in survival among salinities within each cross ($P < 0.05$ based on 95% confidence intervals) are denoted by different superscript letters (i.e., a, b, c). That is, for the same mating (row), mean survival at two different salinities (e.g. 2.5 *vs.* 15 PSU) is significantly different if two mean values have different superscripts.

Type of F1 offspring	Cross	#Replicates (#Clutches)	Salinity (PSU)		
			0	**2.5**	**15**
(**a**) Parental inbred lines	FW_1	14	0.336 ± 0.102^a	0.183 ± 0.074^a	0.023 ± 0.024^b
	FW_2	16	0.619 ± 0.108^a	0.388 ± 0.102^b	0.013 ± 0.018^c
	SW_1	19	0.000 ± 0.000^a	0.154 ± 0.066^b	0.529 ± 0.097^c
	SW_2	11	0.000 ± 0.000^a	0.189 ± 0.090^b	0.550 ± 0.123^c
(**b**) Within-salinity F1 crosses	FW_1xFW_2	10	0.826 ± 0.084^a	0.718 ± 0.109^a	0.164 ± 0.084^b
	FW_2xFW_1	8	0.788 ± 0.104^a	0.513 ± 0.135^b	0.181 ± 0.097^c
	SW_1xSW_2	12	0.042 ± 0.031^a	0.590 ± 0.107^b	0.783 ± 0.090^b
	SW_2xSW_1	9	0.084 ± 0.061^a	0.527 ± 0.128^b	0.655 ± 0.115^b
(**c**) Between-salinity F1 crosses	FW_1xSW_1	12	0.785 ± 0.083^a	0.746 ± 0.091^a	0.788 ± 0.081^a
	SW_1xFW_1	11	0.846 ± 0.076^a	0.842 ± 0.076^a	0.775 ± 0.094^a
	FW_1xSW_2	10	0.883 ± 0.065^a	0.891 ± 0.057^a	0.851 ± 0.072^a
	SW_2xFW_1	8	0.860 ± 0.076^a	0.780 ± 0.104^a	0.798 ± 0.094^a
	FW_2xSW_1	11	0.677 ± 0.104^a	0.741 ± 0.097^a	0.687 ± 0.100^a
	SW_1xFW_2	12	0.709 ± 0.098^a	0.761 ± 0.089^a	0.815 ± 0.083^a
	FW_2xSW_2	10	0.864 ± 0.071^a	0.830 ± 0.084^a	0.749 ± 0.097^a
	SW_2xFW_2	11	0.847 ± 0.082^a	0.863 ± 0.074^a	0.869 ± 0.071^a

Table S2. Maximum likelihood estimates (ML) of probabilities for marginal survival (mean survival across all three salinities, 0, 2.5 and 15 PSU) from hatching to adult. Standard errors (SE) of estimates were obtained by 500 parametric bootstrap resamples.

Type of F1 offspring	**Cross**	**#Replicates** (#clutches)	**ML of Mean Survival ± Bootstrap SE**
(**a**) Parental inbred lines	FW_1	14	0.181 ± 0.051
	FW_2	16	0.336 ± 0.072
	SW_1	19	0.209 ± 0.050
	SW_2	11	0.221 ± 0.062
(**b**) Within- salinity F1 crosses	FW_1xFW_2	10	0.552 ± 0.089
	FW_2xFW_1	8	0.498 ± 0.096
	SW_1xSW_2	12	0.426 ± 0.079
	SW_2xSW_1	9	0.423 ± 0.085
(**c**) Between-salinity F1 crosses	FW_1xSW_1	12	0.774 ± 0.060
	SW_1xFW_1	11	0.814 ± 0.056
	FW_1xSW_2	10	0.865 ± 0.048
	SW_2xFW_1	8	0.812 ± 0.060
	FW_2xSW_1	11	0.700 ± 0.071
	SW_1xFW_2	12	0.762 ± 0.062
	FW_2xSW_2	10	0.810 ± 0.058
	SW_2xFW_2	11	0.852 ± 0.051

Table S3. Differences in maximum-likelihood (ML) estimates of survival (from hatching to adult) in four parental inbred lines and their reciprocal crosses at 2.5 PSU. Differences in ML estimates of survival probability were tested by constructing 95% confidence intervals for mean differences using standard errors (in parentheses) obtained by 500 parametric bootstrap resamples (bold numbers on diagonal are ML estimates of survival for each cross).

Cross	FW_1	FW_2	SW_1	SW_2	FW_1xFW_2	FW_2xFW_1	SW_1xSW_2	SW_2xSW_1	FW_1xSW_1	SW_1xFW_1	FW_1xSW_2	SW_2xFW_1	FW_2xSW_1	SW_1xFW_2	FW_2xSW_2	SW_2xFW_2
FW_1	**0.181**	-0.155 (0.073)	-0.028 (0.059)	-0.040 (0.068)	-0.371 (0.089)	-0.317 (0.098)	-0.245 (0.083)	-0.242 (0.087)	-0.593 (0.067)	-0.633 (0.067)	-0.684 (0.059)	-0.631 (0.069	-0.519 (0.074)	-0.581 (0.069)	-0.629 (0.067)	-0.671 (0.062)
FW_2		**0.336**	0.126 (0.073)	0.114 (0.081)	-0.216 (0.098)	-0.162 (0.107)	-0.091 (0.091)	-0.087 (0.095)	-0.439 (0.079)	-0.478 (0.079)	-0.530 (0.077)	-0.476 (0.082)	-0.364 (0.084)	-0.426 (0.082)	-0.474 (0.079)	-0.517 (0.079)
SW_1			**0.209**	-0.012 (0.065)	-0.343 (0.088)	-0.289 (0.096)	-0.217 (0.078)	-0.214 (0.083)	-0.565 (0.064)	-0.604 (0.062)	-0.656 (0.061)	-0.602 (0.069)	-0.490 (0.072)	-0.552 (0.067)	-0.600 (0.063)	-0.643 (0.060)
SW_2				**0.221**	-0.330 (0.096)	-0.277 (0.104)	-0.205 (0.087)	-0.201 (0.091)	-0.553 (0.075)	-0.592 (0.073)	-0.644 (0.068)	-0.590 (0.075)	-0.478 (0.082)	-0.540 (0.076)	-0.588 (0.074)	-0.631 (0.071)
FW_1xFW_2					**0.552**	0.054 (0.115)	0.125 (0.101)	0.129 (0.109)	-0.223 (0.093)	-0.262 (0.092)	-0.313 (0.090)	-0.260 (0.094)	-0.148 (0.097)	-0.210 (0.097)	-0.258 (0.094)	-0.301 (0.092)
FW_2xFW_1						**0.498**	0.072 (0.110)	0.075 (0.111)	-0.276 (0.102)	-0.316 (0.100)	-0.367 (0.099)	-0.314 (0.104)	-0.202 (0.105)	-0.264 (0.103)	-0.312 (0.098)	-0.354 (0.103)
SW_1xSW_2							**0.426**	0.004 (0.100)	-0.348 (0.088)	-0.387 (0.083)	-0.439 (0.082)	-0.385 (0.089)	-0.273 (0.091)	-0.335 (0.088)	-0.383 (0.082)	-0.426 (0.080)
SW_2xSW_1								**0.423**	-0.352 (0.093)	-0.391 (0.093)	-0.442 (0.088)	-0.389 (0.092)	-0.277 (0.096)	-0.339 (0.090)	-0.387 (0.090)	-0.429 (0.090)
FW_1xSW_1									**0.774**	-0.039 (0.072)	-0.091 (0.068)	-0.037 (0.073)	0.075 (0.078)	0.013 (0.073)	-0.035 (0.070)	-0.078 (0.067)
SW_1xFW_1										**0.814**	-0.010 (0.067)	0.002 (0.074)	0.114 (0.077)	0.052 (0.071)	0.004 (0.067)	-0.039 (0.068)
FW_1xSW_2											**0.865**	0.053 (0.069)	0.166 (0.076)	0.104 (0.068)	0.056 (0.065)	0.013 (0.062)
SW_2xFW_1												**0.812**	0.112 (0.083)	0.050 (0.074)	0.002 (0.073)	-0.041 (0.071)
FW_2xSW_1													**0.700**	-0.062 (0.082)	-0.110 (0.080)	-0.153 (0.079)
SW_1xFW_2														**0.762**	-0.048 (0.072)	-0.091 (0.070)
FW_2xSW_2															**0.810**	-0.043 (0.067)
SW_2xFW_2																**0.852**

Significant ($p < 0.05$) Not significant