Genetica (2009) 137:1–8
DOI 10.1007/s10709-009-9364-0

Rapid response to perturbation of chromosome frequencies in natural populations of *Drosophila robusta*

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Received: 1 November 2008 / Accepted: 8 April 2009 / Published online: 23 April 2009

Abstract Perturbation of gene or chromosome frequencies in natural populations is one of the most powerful ways of demonstrating whether natural selection maintains genetic polymorphism or if other evolutionary forces are at work. Gene arrangement frequencies in two natural populations of *Drosophila robusta* were perturbed multiple times by releasing adult flies with contrasting karyotypes and carefully monitoring post-perturbation presence of hybrids and chromosome frequencies. In all cases, frequencies quickly returned to pre-perturbation levels, and in the following sampling periods, no evidence of the introduced chromosomes was apparent. Analysis of post-perturbation frequency changes included tests for heterogeneity among chromosome arrangements in rates of return to equilibrium values using population admixture analysis. In several cases, significant heterogeneity was detected indicating some form of natural selection was operating. Technical challenges to carrying out perturbation experiments in the wild are also discussed.

Keywords Natural selection · Genetic perturbation · Chromosome · Inversion · *Drosophila robusta*

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Introduction

The extensive evidence for the operation of natural and sexual selection in natural populations has been accumulated using a variety of direct and indirect means (Kingsolver et al. 2001; Hoekstra et al. 2001). Direct experimental manipulations of alternate genotypes or attempts to experimentally perturb gene or chromosome frequencies in natural populations are relatively rare, yet this approach is one of the strongest direct methods to detect selection in nature (Endler 1986). With appropriate controls, if frequencies can be perturbed in a natural population and then return to their previous values, there are few alternate explanations for such results other than the action of some form of natural selection. A variant of this approach was used to detect selective predation on different morphs of *Biston betularia* in field release experiments revealing the basis for industrial melanism in this species (Cook 2003; Majerus 1998; Kettlewell 1961). Other direct approaches of estimating selection in natural populations have demonstrated how pervasive natural and sexual selection can be (Endler 1986, Table 5.1), but surprisingly few cases have involved *Drosophila*. Studies employing selection component analysis have documented sexual and natural selection in natural populations of *Drosophila*, but not by perturbing frequencies (e.g., Anderson et al. 1979; Etges 1996; Ruiz et al. 1986).

There are six published studies where natural populations of *Drosophila* were manipulated by release of particular genotypes that differed in frequency at marker loci or chromosomal arrangements. Early studies with visible mutants (e.g., Spencer 1947) are not included here because of the usual deleterious effects of these phenotypes outside of the laboratory. First, Dubinin and Tiniakov (1946) released thousands of adult *Drosophila funebris* into a

natural population that were homozygous for a relatively uncommon gene arrangement and then resampled populations for several months. Although the flies dispersed rapidly and frequencies declined, they found evidence that heterokaryotypes were overrepresented in these populations, and concluded that natural selection was operating to maintain inversion polymorphism by heterozygote advantage.

Second, natural populations of *Drosophila pseudoobscura* in and around Death Valley, California have been the subject of long term studies of chromosome gene arrangement surveys (Anderson et al. 1991) as well as studies of gene frequency variation. Jones et al. (1981; also discussed in Jones and Parkin 1977) released lab-reared flies homozygous for a rare esterase allele in two isolated desert populations. After release in the spring, allele frequencies in one population returned to pre-perturbation frequencies, and after 58 days, no flies were present. In the following year, this allele was not observed again. In the other population, low fly densities after several weeks prevented further study. The authors concluded that the distribution of gene frequencies in this region was determined by frequent local extinctions and annual recolonization from other source populations.

Third, Barker and East (1980) released lab-reared cactophilic *Drosophila buzzatii* into an isolated population and perturbed gene frequencies at three marker loci. The released flies were homozygous at three unlinked allozyme loci, and introduced into an *Opuntia* cactus patch 13 times by releasing adults and injecting eggs and larvae into fermenting cactus pads. After ca 300 days, allele frequencies returned to preperturbation levels at different rates that the authors concluded could not be explained by migration from adjacent populations. Thus, post-perturbation frequency changes were driven by some form of natural selection. Fourth, this approach was independently repeated in two other populations of *D. buzzatti* in order to carefully estimate migration rates, and similar rates of return in allozyme frequencies were observed in both populations after perturbation (Barker et al. 1989).

Fifth, Turner (1987) performed release experiments with different karyotypes of *D. pseudoobscura* in Bryce Canyon, Utah. About 40 days after release, he found that chromosome frequencies had changed slightly, indicating the released flies had mated with residents. However, there was no evidence that any of the released gene arrangements were still present a year later. Turner (1987) concluded that migration from the much larger local population of flies and population bottlenecking due to harsh winter conditions were probably responsible.

In the sixth study, McKenzie et al. (1994) perturbed allele frequencies at two allozyme loci in Australian wine cellar populations of *Drosophila melanogaster*. These populations had been studied for some time, particularly as one of the loci was *Adh* with particularly well studied biochemical differences between the two most common alleles. These populations use wine casket "seeps" for feeding and breeding thus exposing the flies to high ethanol concentrations (McKechnie and Geer 1993). Populations were monitored for 5 months and frequencies of *Adh* and *Phox* (prophenol oxidase) alleles returned to preperturbation levels at both loci, but at different rates. No migration between cellars was detected, so the authors argued that the return to pre-perturbation frequencies at the *Adh* locus was driven by natural selection operating on the temperature sensitive enzyme activity differences of the two *Adh* alleles involved. Thus, this study and Barker and East (1980) are the most successful perturbation studies in natural populations of *Drosophila*. The latter study is an especially valuable demonstration of natural selection maintaining variation in gene frequencies because the authors explicitly assessed the effects of migration from local populations on rates of gene frequency change at multiple loci.

Why have these types of field experiments been so underused? In animals, one of the most difficult technical problems with releasing different genotypes into nature in order to perturb local frequencies is isolating the effects of local immigration ("swamping") versus possible natural selection in the interpretation of post-perturbation frequency changes. If allele frequencies at unlinked loci return to equilibrium levels at different rates, immigration from adjacent populations can be rejected as an explanation. Also, effects of lab-rearing on the fitness of released individuals is usually unknown, but lab-reared flies are required in order to control gene frequencies as well as control genetic backgrounds of the released individuals. Further, local population densities will also be perturbed with very large releases necessitating smaller releases over an extended period of time (Barker and East 1980; Kettlewell 1973). Nevertheless, genetic perturbation experiments in natural populations are a powerful tool to calibrate laboratory estimates of selection on particular genotypes or chromosome arrangements that form much of our understanding of how genetic variation is maintained in populations (Lewontin et al. 1981; Powell 1997; Lewontin 1974; Endler 1986; Gillespie 1991).

Here, we reanalyze data first published by Levitan (1992) documenting a series of perturbation experiments in natural populations of *Drosophila robusta*. These field studies were performed in the mid-1970s and karyotype frequencies throughout the pre- and post- perturbation phases were documented, but the results were not assessed for the possible effects of local immigration. Chromosome frequencies in two natural populations were perturbed and showed rapid return to pre-manipulation frequencies. Following Barker and East (1980), we used admixture models

(Adams and Ward 1973) to assess rates of migration for different gene arrangements during the post-perturbation periods. In some cases, we provide evidence that local gene arrangement frequencies were resistant to perturbation, consistent with some form of balancing natural selection in these populations of *D. robusta*.

Materials and methods

From 1971 to 1976, six gene arrangement frequency perturbation experiments were performed in Ledgewood and Englewood, New Jersey, ca 64 km apart (Fig. 1). Data for one Englewood experiment were reported in Levitan (1992), but the other results have remained unpublished. In each population, ca 1,500-2,000 mature, adult *D. robusta* were released on 1 day, followed by repeated sampling and assessment of frequency changes for another year or more. Baseline data were collected over several years for each population (prior to the first release) by karyotyping wild caught adults (Levitan 1955). Here, wild males were individually crossed to homokaryotypic females in the lab and at least 7 larvae from these crosses were scored to infer the karyotype of the male. Wild females were despermed in the lab by serial transfer to fresh food vials until no eggs hatched, and then were crossed to stock males for karyotyping. After release, gene arrangement frequencies were determined by karyotyping 10-12 larval progeny of each captured female—these are referred to as egg samples, as well as by karyotyping wild caught adults. Hybrids were identified by the presence of chromosome arrangements derived from the released flies (Table 1). Population sampling before and after the releases was carried out by sweep netting adults over banana baits: attempts were made to make trapping efforts equivalent during each sampling period.

Gene arrangements in natural populations of *D. robusta* were labeled in the order of their discovery, and all are one-step inversions from a "standard" karyotype (Carson 1958). For example, the uninverted left arm of the X chromosome is XL, with inversions XL-1, XL-2, and XL-3. Paracentric inversions are segregating on all chromosome arms of the

Fig. 1 Locations of the two study populations in deciduous woods near Ledgewood and Englewood, New Jersey, USA

Table 1 Frequencies of X chromosome gene arrangement combinations and autosomal gene arrangements of adult *D. robusta* released in both types of experiments

Population	X chromosome						Second chromosome					Third chromosome		
	SS	S1	S2	1S	11	13	22	2L	2L-1	2L-3	2R-1	3R	3R-1	3L-R[b]
Englewood, NJ, May–June, 1969–1971 ($n_X = 468$, $n_a = 604$)[a]	45.3	0.2	1.5	48.1	0.0	0.0	0.4	52.5	30.0	16.6	0.7	96.8	3.2	0.0
Ledgewood, NJ, June 1970–1974 ($n_X = 766$, $n_a = 1{,}003$)[a]	14.8	2.1	3.0	57.7	0.3	0.0	1.8	43.2	17.6	37.1	1.7	90.4	9.6	0.0
"Southern" frequencies—A[c]	0.0	0.0	75.8	0.0	0.0	0.0	24.2	0.0	100.0	0.0	71.0	0.0	100.0	0.0
"Southern" frequencies—B[d]	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
"Northern" frequencies	2.1	2.2	0.0	18.1	72.4	5.2	0.0	13.2	1.4	85.4	0.4	76.8	0.0	23.2

Average population frequencies in the years preceding the releases are also given. See text for details

[a] n_X is the number of X chromosomes and n_a is the number of autosomes sampled

[b] 3L-R is a pericentric inversion restricted to more northern populations (Levitan 1992)

[c] Frequencies of introduced flies in release one and two at Englewood, NJ

[d] Frequencies of introduced flies in release four at Englewood and both releases at Ledgewood, NJ

three metacentric chromosomes except for 3L. X chromosome gene arrangements on the left and right arms are frequently in linkage disequilibrium (Carson 1953) and are labeled in shorthand form. For example, XL.XR-2 is a widespread X chromosome containing gene arrangements XL and XR-2, and is labeled S2, where S refers to the standard gene arrangement. Similarly, XL-1.XR-2 is labeled 12, and XL-2.XR-2 is 22, etc.

Release experiments 1–4 were performed in woods near Englewood, NJ from 1971 to 1975, and releases 1–2 were performed in Ledgewood, NJ in 1974 and 1975. Two different types of releases were carried out at each site. Release of stock populations derived from intercrosses of multiple isofemale lines derived from Myrtle Beach, SC, Alabama, Mississippi, and Louisiana with frequencies of characteristically "southern" gene arrangements permitted observation of the fates of many gene arrangements not found in the New Jersey populations in post-release hybrid progeny (Table 1). The second type of release involved flies derived from a Minnesota population that shared a larger number of gene arrangements in common with the New Jersey populations, but also contained a number of "northern" gene arrangements, unique to higher latitudes (Carson 1958). All lines were cultured in the laboratory to verify gene arrangement frequencies and to increase population sizes before release. No attempt was made to control for potential genetic background/cytoplasm effects of the flies to be released.

We estimated migration rates for the most common X chromosome and gene arrangement on each chromosome arm in post-perturbation samples using population admixture techniques described by Adams and Ward (1973). Declines in post perturbation frequencies due to local introgression should cause frequencies of all arrangements, i.e., X chromosomes, left and right second chromosome arrangements and third chromosome arrangements, to return to pre-perturbation levels at similar rates. We attempted to detect differences in rates of return to pre-release frequencies between chromosome arms by measuring heterogeneity in migration rates for gene arrangement frequencies (Barker and East 1980).

Migration rates, m, were calculated for each chromosomal arrangement as,

$$m = \frac{q_{\text{hyb}} - q_{\text{int}}}{q_{\text{res}} - q_{\text{int}}}$$

where q_{hyb} is the gene arrangement frequency of observed hybrid individuals after release, q_{res} is the frequency of residents prior to release, and q_{int} is the frequency of introduced or released flies into the population (Table 1). The variance of m is,

$$\sigma_m^2 = \frac{1}{(q_{\text{int}} - q_{\text{res}})^2}\left[\sigma_{q_{\text{hyb}}}^2 + m^2\sigma_{q_{\text{res}}}^2 + (1-m)^2\sigma_{q_{\text{int}}}^2\right]$$

and the weighted mean of the migration rates, m_i, for all k gene arrangements is,

$$\bar{m} = \sum_{i=1}^{k}\left[\frac{m_i}{\sigma_{m_i}^2}\Big/\left(\sum_{i=1}^{k}\frac{1}{\sigma_{m_1}^2}\right)\right].$$

To test for heterogeneity among migration rates, the appropriate Chi square is,

$$\chi_{(k-1)}^2 = \sum_k \frac{(m_i - \bar{m})^2}{\sigma_{m_i}^2}.$$

All equations are from Adams and Ward (1973).

Results and discussion

In all cases, post-perturbation frequencies returned to equilibrium frequencies in several months, despite appreciable frequencies of hybrid larvae detected in the progeny of both captured resident and released females (Figs. 2, 3). Released adults were captured after 1-2 weeks indicating reasonable survival of lab-reared flies in the wild, although the 1975 release at Englewood was far less successful. For example, 2 weeks after the 1971 release at Englewood, 27.8% of females and 43.4% of males captured were released adults. Similar rates of return to baseline frequencies occurred at both sites whether the released flies were derived from southern or northern populations (Figs. 2, 3). Frequencies of hybrid adults were low, ranging from 5 to 12%, so few hybrid progeny reached adulthood. By the following year, there was little evidence of any segregating foreign gene arrangements in either population.

Analysis of post-perturbation frequencies revealed instances of significant heterogeneity in the rate of return to baseline frequencies among X chromosomes and autosomal chromosome arms (Tables 2, 3). Almost all egg samples showed significant heterogeneities, presumably due to different rates of insemination of wild caught females by released males and released females by wild males. Lower introgression rates of 2L-1 than the other chromosomes in the July 1–8 adult sample from Release 1 at Englewood ($P < 0.1$), the August 31–October 23 adult ($P < 0.05$), and May 1976 egg ($P < 0.001$) samples from Release 2 at Ledgewood suggest natural selection was operating on

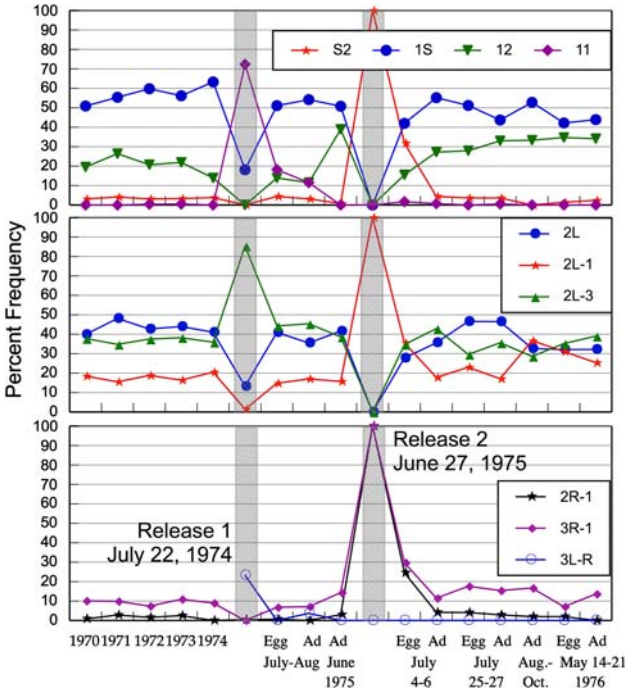

Fig. 3 Results of a parallel perturbation experiment in Ledgewood, New Jersey involving two releases. See Fig. 2 for details

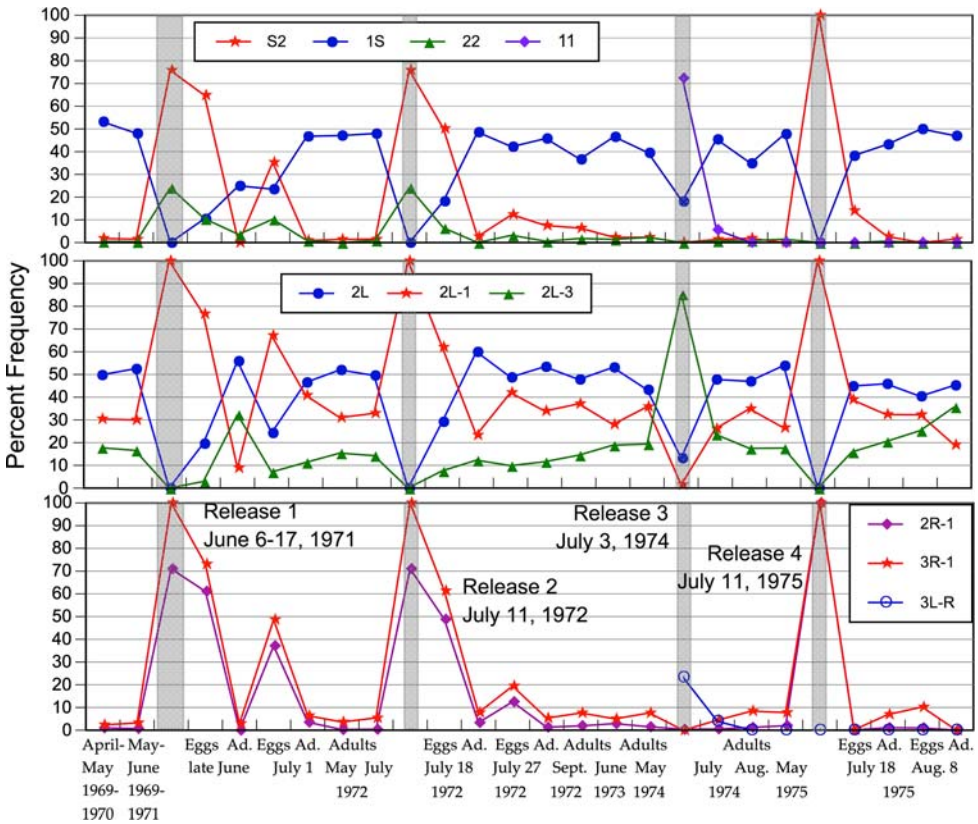

Fig. 2 Gene arrangement frequency changes before and after four perturbation experiments in Englewood, New Jersey. "Eggs" refers to frequencies of 10–12 offspring of each wild-caught (non-release) female, and ad. or adults refers to arrangement frequencies of captured (non-release) adults. *Gray bars* indicate when flies were released

Table 2 Estimates of Englewood, New Jersey weighted migration rates, *m*, and their ranges following the release of (A) "southern" karyotypes (release 1, 2, 4) and (B) "northern" karyotypes (release 3) of *D. robusta*

Sampling date	*n*	*m*	Range	X^2	*P*
A					
Release 1. June 6–17, 1971					
June 25–27 egg	302	0.2612	0.141–0.618	43.67	<0.001
June 25–27 adult	28	1.0133	0.866–1.303	14.26	<0.05
July 1 egg	70	0.5392	0.482–0.657	3.57	
July 1–8 adult	71	0.9520	0.846–1.008	9.37	<0.1
April 30–May 5, 1972 adult	153	1.0010	0.986–1.017	1.64	
June 16–July 3, 1972 adult	129	0.9983	0.959–1.004	3.53	
Release 2. July 11, 1972					
July 18–19 egg	216	0.4245	0.314–0.763	53.98	<0.001
July 18–19 adult	47	1.0100	0.969–1.143	2.32	
July 27 egg	798	0.8526	0.829–0.974	19.02	<0.01
July 27 adult	206	0.9919	0.888–1.017	3.28	
September 22–27 adult	80	0.9706	0.934–0.979	1.77	
May 30–June 20, 1973	121	0.9862	0.965–1.073	5.22	
Release 4. July 11, 1975					
July 18–20 egg	826	0.8781	0.0–0.930	12.07	<0.025
July 18–August 1 adult	356	0.9801	0.400–1.010	8.43	
August 8 egg	50	1.0328	0.0–1.085	3.92	
August 8 adult	21	0.9930	0.0–1.010	2.17	
B					
Release 3. July 3, 1974					
July 10–12 egg	264	0.9064	0.793–0.918	0.65	
July 10–12 adult	103	0.9043	0.091–0.935	5.45	
August 16–19 egg	99	0.9987	0.878–4.273	1.21	
August 16–19 adult	83	1.0020	0.727–1.099	0.39	
May 14–22, 1975 adult	51	1.0111	0.928–1.455	0.22	

Significant X^2 values indicate heterogeneity in levels of admixture for chromosomal arrangements in egg samples ("egg") of wild, inseminated females and wild–caught adults. *n* is the number of adults or larvae karyotyped

Table 3 Estimates of Ledgewood, New Jersey weighted migration rates, *m*, and their ranges following the release of (A) "northern" karyotypes (release 1) and (B) "southern" karyotypes (release 2) of *D. robusta*

Sampling date	*n*	*m*	Range	X^2	*P*
A					
Release 1. July 22, 1974					
July 29–August 1 egg	88	0.8034	0.095 to 1.000	7.97	
July 29–August 1 adult	71	0.7217	−0.190 to 0.847	73.80	<0.001
June 20–27, 1975 adult	84	1.0072	−0.048 to 1.776	9.13	
B					
Release 2. June 27, 1975					
July 4–6 egg	431	0.7166	0.429 to 0.772	9.95	<0.05
July 4–6 adult	155	0.9341	0.699 to 1.033	8.10	
July 25–27 egg	343	0.9724	0.717 to 1.001	12.61	<0.025
July 25–27 adult	143	0.9857	0.771 to 1.014	5.95	
August 31–October 23 adult	25	0.9220	0.751 to 1.023	10.06	<0.05
May 14–21, 1976 egg	98	0.9988	0.817 to 1.084	21.09	<0.001
May 14–21, 1976 adult	30	1.0191	0.877 to 1.044	7.87	

Significant X^2 values indicate heterogeneity in levels of admixture for chromosomal arrangements. Both egg samples of wild, inseminated females and wild–caught adults were included. *n* is the number of adults or larvae karyotyped

post-perturbation frequencies (Figs. 2, 3; Tables 2, 3). The significant result for the July 29–August 1, 1974 adult sample at Ledgewood was anomalous (Table 3A), and could not be due to introgression as this sample was made just a week after the release: egg to adult development time is at least 2 weeks in the laboratory (Etges 1989) and

probably longer in the wild. Also, it is not clear whether the significant heterogeneity observed in the Ledgewood Release 2, May 1976 post-winter frequencies was due selection against these introduced arrangements or because 2L-1 (Fig. 3) was responding to seasonal temperature changes—2L-1 is a "warm" adapted gene arrangement (Etges 1989; Levitan 1992; Etges and Levitan 2008). Overall, there was evidence for natural selection, rather than swamping from local populations, operating on some of the changes in post-perturbation frequencies.

These genetic perturbation experiments represent a rare example of introgression of lab-reared *Drosophila* into natural populations in attempts to directly detect natural selection in the wild. The most successful experiment with *D. buzzatii* carefully documented the role of natural selection during the post-perturbation phase based on three unlinked allozyme loci by disentangling natural selection from swamping by migration from local populations (Barker and East 1980). The wine cellar perturbation experiments also controlled for immigration effects (McKenzie et al. 1994), but also relied on extensive background knowledge of the *Adh* enzyme polymorphism in *D. melanogaster*. The other release experiments with gene arrangements involving *D. funebris* (Dubinin and Tiniakov 1946) and *D. pseudoobscura* (Turner 1987) were more similar to the New Jersey *D. robusta* results (Figs. 2, 3).

These studies offer some insight into the strength of natural selection in the wild, as well as enabling the technical difficulties of these experiments to be identified. For *D. robusta*, despite positive evidence that released adults had mated, with significant numbers surviving for several weeks in the wild, only small numbers of hybrid adults were captured. Even if released flies had originated from these New Jersey populations and manipulated in the lab to increase frequencies of less common gene arrangements to eliminate genetic background effects, other potential difficulties inhibiting increased introgression with wild flies seem difficult to surmount. There is no evidence for sexual isolation between populations of *D. robusta* (Arbuckle and Etges, unpublished data) so low frequencies of hybrids and the rapid declines in chromosome frequencies after perturbation were more likely due to low hybrid fitness or perhaps nuclear-cytoplasmic incompatibilities between the released and resident flies. In retrospect, the limited success of these introgression experiments likely includes; (1) the effects of lab rearing on release flies, (2) the unknown number of natural breeding sites (Carson and Stalker 1951), (3) the effects of releasing large numbers of adults on local population densities, and (4) the limited number of possible hybrid generations per growing season due to the long preadult and adult life spans of *D. robusta* (Etges 1989). In order to insure higher initial introduced chromosomal frequencies so that post-perturbation dynamics can be more carefully assessed, these manipulations should be repeated with flies derived from the population at the release point over longer time periods given the long generation times of this species, perhaps in more southerly populations where the growing season is longer. Nevertheless, the post-perturbation frequency dynamics in these New Jersey populations (Figs. 2, 3) suggests that some chromosome arrangements returned to equilibrium frequencies for reasons other than local introgression, i.e., natural selection.

Acknowledgments We thank J. R. Powell and M. E. Turner for discussions and insights concerning the history of *Drosophila* release experiments and two anonymous reviewers for valuable comments on the manuscript.

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