Environmental Entomology, 52(4), 2023, 740–749
https://doi.org/10.1093/ee/nvad064
Advance Access Publication Date: 17 July 2023
Research

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY

OXFORD

Population Ecology

Seasonal differences in the timing of flight between the invasive winter moth and native Bruce spanworm promotes reproductive isolation

Jeremy C. Andersen[1,*], Nathan P. Havill[2], Jennifer L. Chandler[1], George H. Boettner[1], Brian P. Griffin[1,3], Joseph S. Elkinton[1]

[1]Department of Environmental Conservation, University of Massachusetts Amherst, Amherst, MA 01003, USA, [2]USDA-Forest Service, Northern Research Station, Hamden, CT 06514, USA, [3]Present Address: Vilceck Institute of Graduate Biomedical Sciences, Grossman School of Medicine, New York University, New York, NY 10012, USA *Corresponding author, mail: jcandersen@umass.edu

Subject Editor: Darrell Ross

Received on 16 December 2022; revised on 16 May 2023; accepted on 23 June 2023

The European winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae), was accidentally introduced to North America on at least 4 separate occasions, where it has been hybridizing with the native Bruce spanworm, *O. bruceata* Hulst, at rates up to 10% per year. Both species are known to respond to the same sex pheromones and to produce viable offspring, but whether they differ in the seasonal timing of their mating flights is unknown. Therefore, we collected adult male moths weekly along 2 transects in the northeastern United States and genotyped individuals using polymorphic microsatellite markers as males of these 2 species cannot be differentiated morphologically. Along each transect, we then estimated the cumulative proportions (i.e., the number of individuals out of the total collected) of each species on each calendar day. Our results indicate that there are significant differences between the species regarding their seasonal timing of flight, and these allochronic differences likely are acting to promote reproductive isolation between these 2 species. Lastly, our results suggest that the later flight observed by winter moth compared to Bruce spanworm may be limiting its inland spread in the northeastern United States because of increased exposure to extreme winter events.

Key words: hybrid zone, reinforcement, pre-zygotic isolation, allochrony, *Operophtera brumata*

Introduction

The introduction of non-native invasive species into naïve habitats has the potential to result in catastrophic changes in ecosystem function and resilience (Mack et al. 2000), and can result in the reductions or even extinctions of native species (Brockerhoff et al. 2006, Murrell et al. 2011, Gallien and Carboni 2017). Studying invasion events can provide important opportunities to observe evolutionary and ecological processes (Alpert 2006, Prentis et al. 2008, Ward et al. 2008, Jones and Gomulkiewicz 2012), and these have long been viewed as "natural laboratories" (Herder et al. 2012, Paillex et al. 2017, Diaz-Puente et al. 2020, Ejsmont-Karabin et al. 2020, Valencia-Montoya et al. 2020). One way in which species invasions can alter evolutionary trajectories and ecological interactions is through hybridization (Havill et al. 2012, Zemanova et al. 2017, Correa et al. 2019, Cordeiro et al. 2020, Popovic et al. 2021, Andersen et al. 2022). Of particular interest has been the role of invasive species in driving a process known as hybridization to extinction (Hinton 1975, Levin et al. 1996, Rhymer and Simberloff 1996, Wolf et al. 2001, Prentis et al. 2007, Todesco et al. 2016, Zemanova et al. 2017), wherein native species can either be lost to extinction or to a "hybrid swarm" (Allendorf et al. 2001). Conservation managers have been primarily focused on the role of hybridization in driving the loss of native biodiversity and disrupting ecosystem function, but it has recently been suggested that hybridization events can also limit the spread or even drive the local extinction of the invasive species (Andersen et al. 2022). As such, hybridization could present a form of biotic resistance for the native communities (sensu Levine et al. 2004, Alpert 2006, Gallien and Carboni 2017).

This type of "biotic resistance" could very well be promoted via the reinforcement concept of sympatric speciation (Fisher 1930, Dobzhansky 1940, Spencer et al. 1986, Butlin 1987, Liou and Price 1994, Servedio and Noor 2003). Under reinforcement theory,

pre- and postzygotic barriers to geneflow establish or maintain species identities through processes such as physical incompatibilities, hybrid infertility, and reduced hybrid fitness (Servedio and Noor 2003). In their excellent review, Servedio and Noor (2003) note that reinforcement has been demonstrated empirically and theoretically in numerous systems (for recent examples see Groh and Cronk 2020, O'Brien et al. 2021, Punzalan et al. 2021, Teixeira et al. 2021), but note that there is a lack of temporal studies that examine reinforcement over multiple generations (but see Pfennig and Simovich 2002, Pfennig et al. 2016). In addition, there have been few studies that have examined reinforcement in the context of species invasions (but see Irimia et al. 2021, (Sirkia et al. 2018) though it has been discussed in a biological control context (see Fischer et al. 2015), which in many regards is very similar to a species invasion context (Schulz et al. 2021).

The invasion of the northeastern United States by the European winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae) presents an excellent opportunity to study ecological and evolutionary changes associated with the real-time formation of a hybrid zone, including the factors that establish or promote reinforcement of reproductive isolation. For over ten years, this system has been studied in an effort to understand the ecological effects of this destructive tree defoliator and to implement a successful biological control program (Elkinton et al. 2010, 2011, 2014, 2015, 2021). Early in those studies, it was noted that winter moth was hybridizing with a native congener, the Bruce spanworm, *O. bruceata* Hulst (Elkinton et al. 2010, 2014). Subsequently, it was determined that multiple generations of hybrids (i.e., F_1, F_2, and backcrosses) could be detected in the field (Havill et al. 2017), and that the hybrid zone was clinal in nature persisting under a tension model (Andersen et al. 2022). Tension hybrid zones are predicted to be formed when dispersal is limited, when there are large differences in the relative abundances of the 2 hybridizing species, and when hybrid individuals have reduced fitness (Gay et al. 2008)—the latter being a factor that is also predicted to promote reinforcement.

Several pre-zygotic barriers to hybridization may exist in this system. For example, morphological differences in the genitalia of winter moth and Bruce spanworm have long been known (Troubridge and Fitzpatrick 1993, Elkinton et al. 2010, Griffin et al. 2020), and it is possible that these differences could promote reinforcement if they deter mating (i.e., "lock-and-key" incompatibilities), and/or if outcrossing results in physical damage to the genitalia. Outcrossing could further reduce an individual's fitness if it results in increased exposure to predators, increased expenditure of limited energy stores, or if outcrossing results in mistaken cues for successful mating (Servedio and Noor 2003). While hybrid individuals are commonly observed in the winter moth × Bruce spanworm system (Andersen et al. 2019), laboratory experiments have found that females of both species produce fewer viable eggs when outcrossed compared to females mated with males of their same species (Havill et al. 2017), indicating that outcrossing has a negative fitness cost.

An additional pre-zygotic barrier to hybridization that might exists in this system is allochronic differences in the timing of mating flights. Species of insects can develop different timings for mating activities as a result of reinforcement (Cardé and Baker 1984), and these differences can be both related to the timing of the day (i.e., diel) and/or to differences in time of year (i.e., seasonal). Rapid changes in both diel (Fergus et al. 2011) and seasonal (Scriber and Ording 2005, Santos et al. 2007) timing can occur as a result of reinforcement, and therefore allochronic partitioning might be present (or could develop) in the winter moth × Bruce spanworm system. In regard to potential seasonal differences, both species are known to be active in the winter months, though the exact timing of these flights for each species in North America have yet to be recorded. To document whether there are seasonal differences in the timing of flight between these 2 species, we collected male moths weekly or bi-weekly along 2 transects throughout the suspected seasonal flight windows of both winter moth and Bruce spanworm in the northeastern United States. We then estimated cumulative emergence data, and fit generalized linear models to determine whether there are differences in flight time between the species, transects, and across years of study in Massachusetts and Connecticut, and compare these results to weather data from each collection year to gain insights into factors that could prematurely terminate flight.

Materials and Methods

Collection and Genotyping Moths

Collection and genotyping methods for all samples included in this study were reported in Andersen et al. (2022), and are summarized here. Briefly, adult male moths of both species were collected using pheromone-baited lures (Great Lakes IPM, Vestaburg, MI) placed within plastic bucket traps (Gempler's Inc., Janesville, WI) that each contained an insecticide strip following the procedure presented in Elkinton et al. (2010, 2011). Previously, we established 2 transects that span the winter moth × Bruce spanworm hybrid zone in the northeastern United States: 1 in Massachusetts along Route 2 and 1 in Connecticut along Route 1 (Fig. 1). At each site, trapping began at the end of October and concluded in the beginning of January (incorporating the period in which we have previously observed flight in the region). Along the Massachusetts transect, we sampled traps weekly in 2017 and bi-weekly in 2018, while along the Connecticut transect, we sampled traps weekly in 2016, 2017, and 2018. The number of moths collected at each trap is presented in Table 1, and complete collection information including the number of moths per collection event is provided in Supplementary Table S1.

During each collection event, all adult male moths were removed from the traps, the total number of moths was recorded, and the moths were transferred into glassine envelopes (Uline Inc., Pleasant Prairie, WI) before being stored at −80°C for subsequent molecular analyses. As morphological-based approaches are incapable of distinguishing our focal species (Griffin et al. 2020), to classify individual male moths as either winter moth, Bruce spanworm, or hybrids, up to 20 individuals from each collection event were haphazardly selected and total genomic DNA was extracted using the EZNA Tissue DNA extraction kit (Omega Bio-Tek; Norcross, GA). Prior to extraction, the wings and uncus of each moth were removed and saved as vouchers, and the remaining body contents were homogenized. Eleven polymorphic microsatellites were then amplified using the protocols presented in Havill et al. (2017). PCR products were genotyped at the DNA Analysis Facility on Science Hill at Yale University, and alleles were scored using the microsatellite plugin in GENEIOUS v. R11 (https://www.geneious.com). The microsatellite genotype scores were then used to estimate the probability of assignment (Z) of each moth as either a pure Bruce spanworm, a pure winter moth, or a hybrid (either as an F_1, F_2, or a backcross) using the Bayesian-assignment program NewHybrids v 1.1 b3 (Anderson and Thompson 2002, Anderson 2008). In an effort to reduce assignment errors due to the possibility that individual moths from 1 yr could be the parents of moths collected in the subsequent year (which could influence the assignment estimates), a separate dataset for each year and for each transect was analyzed independently 4 times using uniform priors, random starting seeds, burn-in periods of 100,000 generations, a post-burn-in runtime of

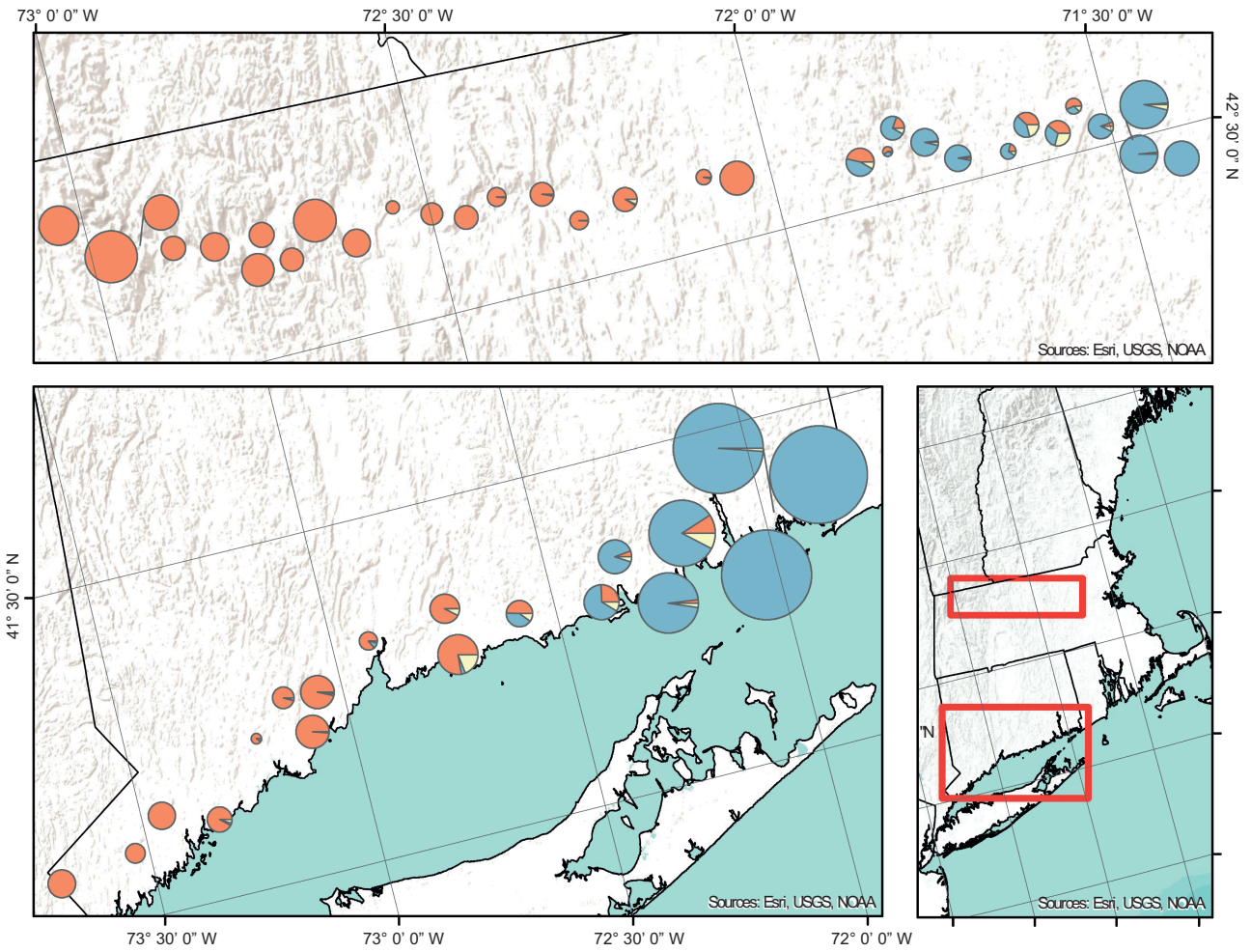

Fig. 1. Locations of the Massachusetts transect (top) and the Connecticut transect (bottom left), in relation to the northeastern United States (bottom right). The sizes of individual traps are drawn proportional to the average number of winter moth (blue), Bruce spanworm (orange), and hybrid (yellow) individuals collected at that location across study years.

1,000,000 generations, and the assignment scores from each run were then averaged.

Estimating Differences in Seasonal Timing of Flight

All analyses of seasonal timing were performed in R v. 4.2.1 (R Core Team 2022). For each collection event, the total number of moths collected in each trap was multiplied by the proportion of moths classified to each species category (i.e., as either Bruce spanworm, winter moth, or hybrids) based on the microsatellite genotyping results to estimate the total number of individuals of each species-class present during each collection event. The dataset was then subdivided by year, transect, and species, and filtered so that only traps with ≥10 moths of a species-transect-year combination were included. We removed traps with a total trap catch of <10 moths for a focal species due to concerns that the proportional analyses would be highly influenced by stochasticity associated with small sample sizes. For each species at each collection event, the cumulative proportion of moths was calculated by summing the estimated number of that species collected during the collection event and all previous collection events and dividing it by the total estimated number at that trap for the entire season. Differences in cumulative proportions were examined globally across all years within a transect using generalized linear models (glm) via binomial logistic regression with a quasi-binomial fit and a logit-link function to account for the proportional nature of the datasets, with cumulative proportion as the response variable and species and calendar days (and their interaction) as well as year as fixed effects. Note: as the flight window for our focal species runs from October of 1 yr into January of the next year, calendar dates were modified so that collection events occurring after January 1st had 365 days added to them. For example, a collection that occurred on January 10th would be reported as occurring on day 375. Post-hoc analyses were then performed for each species-year combination within a transect using the same glm approach above with proportion of adults as the response variable and with only calendar days as a fixed effect and were used to estimate the average dates for 3 flight categories: initiation, peak, and the tail-end (i.e., the dates at which 10%, 50%, and 90% of the population had flown, respectively) using the "dose.p" function in the package "MASS". Differences between years and species were then compared based on estimates of 95% confidence intervals for each of the categories above.

Environmental Variables Associated With Seasonal Timing of Flight

To explore whether flight for either species might be terminated by the onset of subzero temperatures (O'Donnell and Groden 2017)

Table 1. (a) Seasonal totals for Bruce spanworm, winter moth, and hybrid individuals collected from traps along the Massachusetts Route 2 transect. (b) Seasonal totals for Bruce spanworm, winter moth, and hybrid individuals collected from traps along the Connecticut Route 2 transect

	Bruce spanworm			Winter moth			Hybrids		
Trap	2016	2017	2018	2016	2017	2018	2016	2017	2018
(a)									
T00		495	90		0	0		0	0
T01A		258	37		0	0		0	0
T01B		180	38		1	0		0	0
T01C		400	41		2	0		0	0
T01D		811	234		0	0		0	0
TJB		337	52		0	0		0	0
T02A		236	51		0	0		0	0
T02B		617	19		0	0		0	0
T02C		171	29		0	0		0	0
T02D		157	95		0	0		0	0
T03		54	11		0	0		0	0
T03A		160	15		0	0		0	0
T03B		159	61		0	0		0	0
T04		91	50		0	0		2	0
T04A		174	31		4	1		0	0
T04B		95	42		1	0		0	1
T05		153	51		2	2		12	3
T05B		72	13		3	0		0	0
T06		371	39		1	1		3	0
T07		108	32		97	51		21	2
T08		19	3		10	1		2	1
T09		24	26		119	38		16	1
T10		3	4		210	69		7	3
T11		6	1		172	96		6	0
T13		16	3		49	16		6	3
T14		49	54		65	40		37	13
T15		70	29		60	17		57	12
T16		41	15		21	12		10	2
T17		11	3		165	49		15	1
T18		3	10		544	404		27	4
T19		4	7		401	164		1	0
T20		0	0		238	313		0	0
(b)									
CT01	227	197	83	0	0	0	0	0	0
CT02	79	115	33	1	0	0	0	0	0
CT03	197	202	64	1	0	0	0	0	0
CT04	127	142	101	5	6	0	0	14	2
CT05	87	0	7	11	0	0	0	0	0
CT06	65	124	89	5	0	0	0	7	0
CT07	266	358	0	0	0	0	0	5	0
CT08	80	327	206	3	8	0	0	7	0
CT09	57	9	167	4	14	0	1	2	4
CT10	425	278	152	24	10	6	39	112	8
CT11	343	167	58	0	3	0	26	15	4
CT12	131	69	46	129	58	20	14	20	7
CT13	92	73	44	136	201	178	29	21	20
CT14	21	16	6	215	202	278	25	12	0
CT15	42	6	5	563	715	1062	43	18	5
CT16	129	99	42	389	965	898	90	102	13
CT17	0	14	0	2532	2387	0	23	23	0
CT18	0	0	0	3529	1518	837	23	29	0
CT19	0	19	1	1432	2398	2169	14	0	0

or heavy snow fall (Peterson and Nilssen 1998) we obtained daily maximum temperature (tmax) and daily snow water equivalent (swe) and data for each trap location from 1 January 2016 through 28 February 2019 using the DayMet "single pixel extraction tool" (Thornton et al. 2020).

Results

Collection and Genotyping Moths

Genotypes obtained in Andersen et al. (2022) from 32 locations in Massachusetts and 19 locations in Connecticut, totaling 6,138

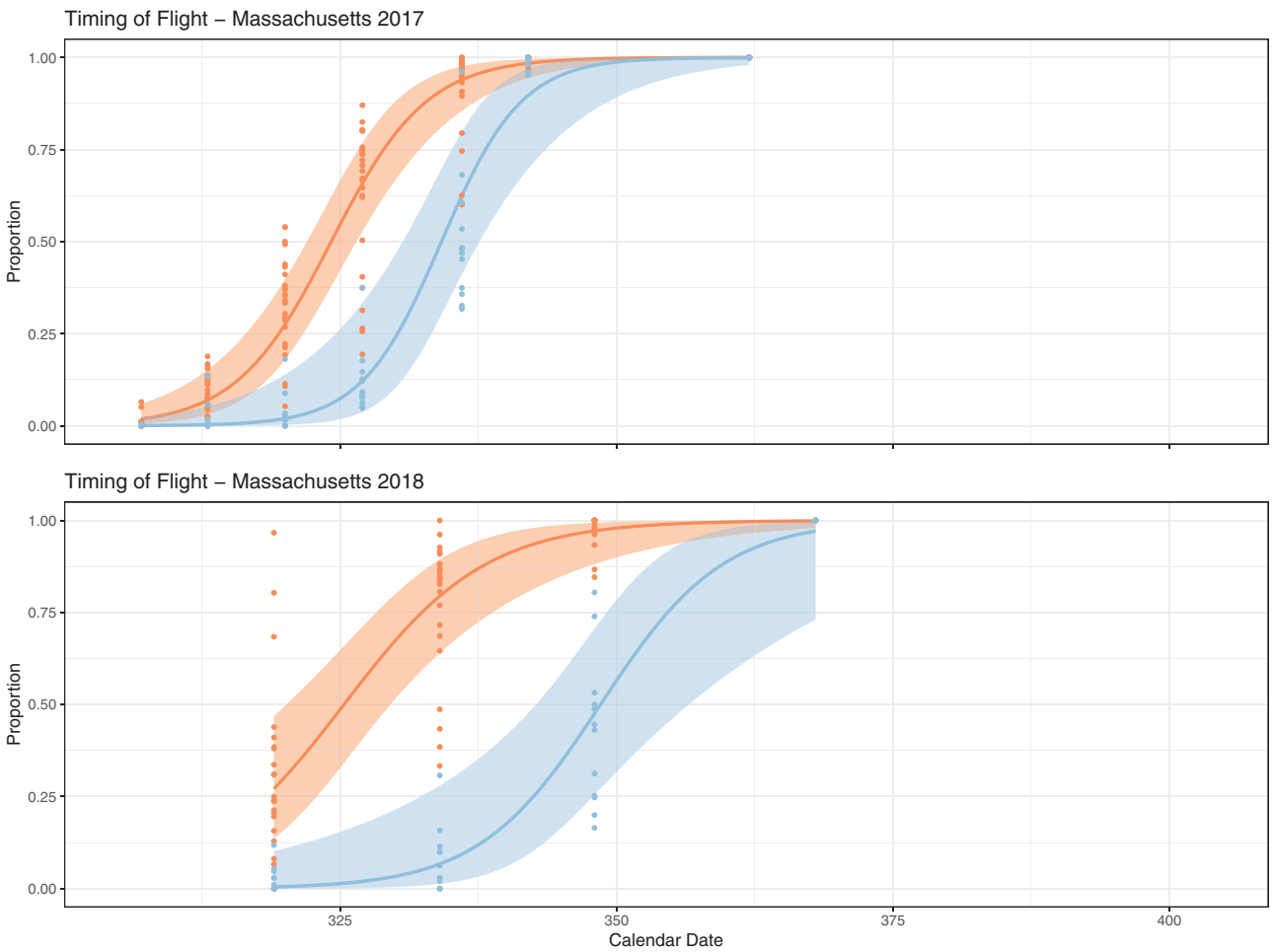

Fig. 2. Cumulative proportion charts representing the timing of flight (calendar day) along the Massachusetts transect in 2017 (top) and 2018 (bottom) for winter moth (blue) and Bruce spanworm (red).

individual moths were used to calculate the overall abundance for each species at each trap. Genotype success was quite high across sampled years, and while not reported in Andersen et al. (2022), on average 10.48 loci ± 0.02 (out of a possible 11) were included to estimate species status for each individual. The total estimates for the abundance of each species in Massachusetts are presented in Table 1a and for Connecticut in Table 1b. Because of the dramatic decrease in the number of locations from which ≥10 hybrid individuals were collected across both transects in 2018 ("Table 1a and b), we did not directly estimate the timing of flight for hybrids, though we hope to do so in future studies.

Estimating Differences in the Seasonal Timing of Flight

Analyses based on data from the Massachusetts transect indicated that there were significant differences in the timing of flight between winter moth and Bruce spanworm (SS = 5.59, df = 1, $P < 0.0001$), with calendar dates (SS = 50.18, df = 1, $P < 0.0001$), year (SS = 1.14, df = 1, $P < 0.0001$), and the interaction between species and date (SS = 0.03, df = 1, $P = 0.0079$) all being significant predictors for cumulative proportions. Results are presented graphically in Fig. 2, and estimates for the timing of each flight stage for each species and year are presented in Table 2. Post-hoc analyses indicated that in both survey years there were significant differences in the timings of all flight categories between winter moth and Bruce spanworm based on non-overlapping 95% confidence intervals. Analyses based on data from the Connecticut transect indicated that there were significant differences in the timing of flight between winter moth and Bruce spanworm (SS = 1.85, df = 1, $P < 0.0001$), with calendar dates (SS = 74.24, df = 1, $P < 0.0001$), year (SS = 4.06, df = 2, $P < 0.0001$), and the interaction between species and date (SS = 0.06, df = 1, $P < 0.0001$) all being significant predictors for cumulative proportions. Results are presented graphically in Fig. 3, and estimates for the timing of each flight stage for each species and year are presented in Table 2. Post-hoc analyses indicated that in both survey years there were significant differences in the timings of all flight categories between winter moth and Bruce spanworm based on non-overlapping 95% confidence intervals.

Environmental Variables Associated With Seasonal Timing of Flight

Daily maximum temperatures (tmax) and snow water equivalent (swe) values for the Massachusetts transect are presented in Supplementary Fig. S3 and for the Connecticut transect in Supplementary Fig. S4.

Discussion

Here we explored allochronic differences in the seasonal timing of flight for 2 hybridizing species, 1 native to North America (the Bruce

Table 2. Comparisons of the seasonal timing of flight along the Massachusetts (MA) and Connecticut (CT) transects for populations of winter moth and Bruce spanworm. The average calendar date, along with 95% confidence intervals, are presented for 3 flight categories including the initiation of flight (i.e., the point at which 10% of the population had flown), peak flight (i.e., the point at which 50% of the population had flow), and the tail end of flight (i.e., the point at which 90% of the population had flow)

		Massachusetts			Connecticut		
	Year	Initiation (0.1)	Peak (0.5)	Tail (0.9)	Initiation (0.1)	Peak (0.5)	Tail (0.9)
Winter moth	2016	—	—	—	332.73	339.27	345.82
		—	—	—	(331.65, 333.80)	(338.60, 339.95)	(344.73, 346.91)
	2017	326.2	334.16	342.11	332.64	344.36	356.08
		(324.22, 328.18)	(332.95, 335.38)	(340.17, 344.05)	(330.82, 334.47)	(343.26, 345.47)	(354.24, 357.92)
	2018	336.34	348.54	360.68	337.35	350.67	363.99
		(332.99, 339.69)	(346.31, 350.77)	(356.58, 364.78)	(334.86, 339.85)	(349.00, 352.38)	(360.25, 367.74)
Bruce spanworm	2016	—	—	—	329.39	336.1	342.82
		—	—	—	(328.50, 330.27)	(335.57, 336.64)	(341.94, 343.70)
	2017	314.71	324.18	333.65	328.21	336.01	343.82
		(313.75, 315.67)	(323.57, 324.79)	(332.63, 334.67)	(327.02, 329.40)	(335.30, 336.72)	(342.75, 344.88)
	2018	311.24	325.33	339.41	330.04	339.55	349.06
		(308.12, 314.36)	(323.72, 326.94)	(336.88, 341.94)	(328.74, 331.34)	(338.81, 340.29)	(347.82, 350.30)

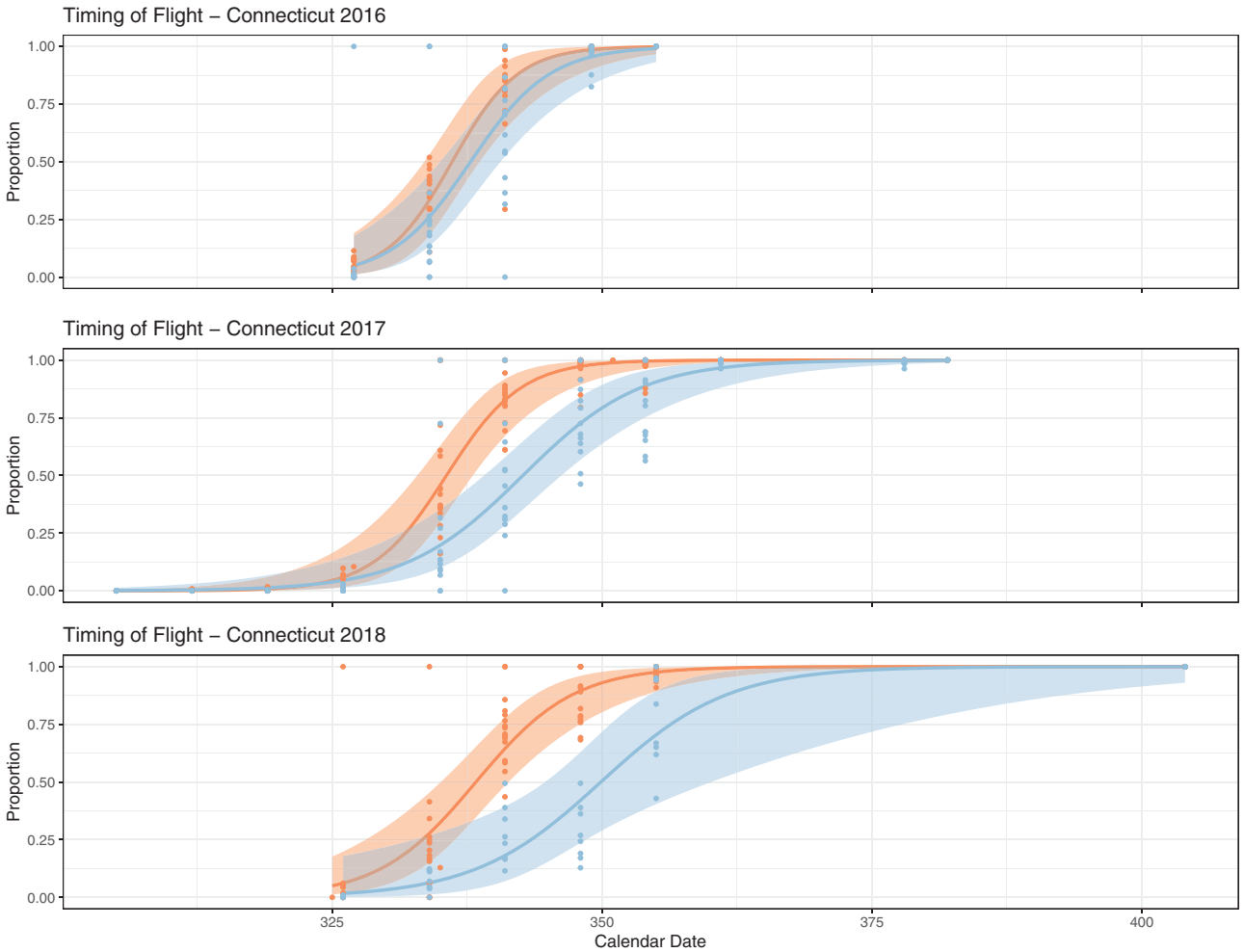

Fig. 3. Cumulative proportion charts representing the timing of flight (calendar day) along the Connecticut transect in 2016 (top), 2017 (middle), and 2018 (bottom) for winter moth (blue) and Bruce spanworm (red).

spanworm) and 1 invasive from Europe (the winter moth). Given the extensive documentation of hybridization in this system, it is not surprising that we found that the seasonal timing of flight for both species broadly overlap (Fig. 4). However, despite this overlap we found significant differences in the timing of all observed flight stages, with winter moth flight stages occurring on average 17 days

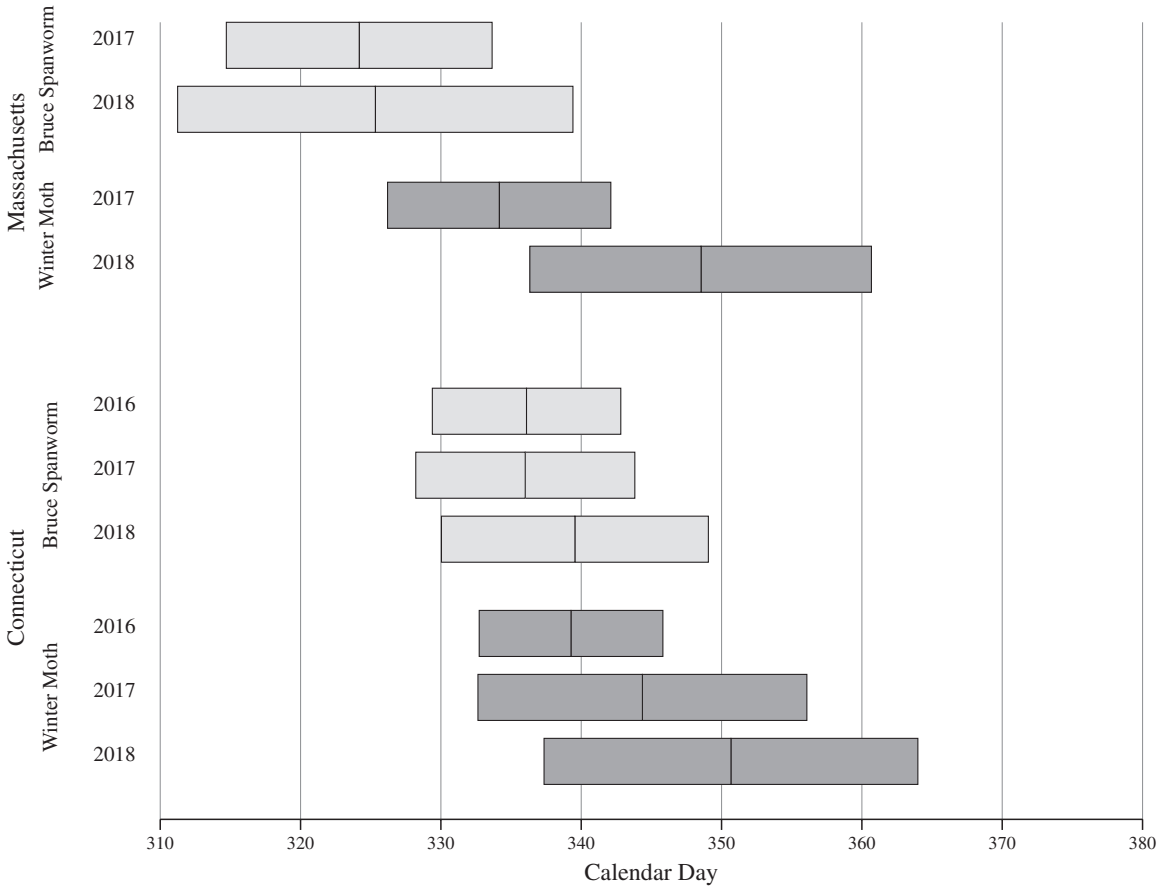

Fig. 4. Summary of flight stage timing for Bruce spanworm (light gray) and winter moth (dark gray) along the Massachusetts (top) and Connecticut (bottom) transects for each year of study. Boxes are drawn so the left border represents the average values for initiation of flight, the middle line represents the average values for peak flight, and the right border represents the average values for the tail-end of flight as per Table 2.

later than those for Bruce spanworm in Massachusetts (for comparison, here we find that the average winter moth flight window from initiation through the tail-end was 20.125 days ± 4.22 days, and the average Bruce spanworm flight window was 23.55 ± 4.62 days). Similar differences were observed in Connecticut where winter moth flight was on average 7 later than those for Bruce spanworm in Connecticut (where the average winter moth flight window from initiation through the tail-end was 21.06 days ± 4.09 days, and the average Bruce spanworm flight window was 16.02 ± 1.63 days). While interspecific differences in male genitalia are present in this system (Griffin et al. 2020), these differences do not prevent mating (Havill et al. 2017), and both species are known to respond to the same sex pheromone (Elkinton et al. 2010, 2011). Therefore, we believe that the observed allochronic differences could provide the natural variation necessary upon which reinforcement could act to prevent a hybrid swarm from developing (Allendorf et al. 2001, Harrison and Larson 2014), and are in part responsible for the creation of a clinal hybrid zone in this system (Andersen et al. 2022).

In addition to possibly reducing hybridization rates—a trend we observed over a 12-yr period along the Massachusetts transect where hybridization rates decreased from a peak of ~10% in 2010 to ~3% in 2018 (Andersen et al. 2022)—reinforcement may also be acting to limit the spread of winter moth into the interior portions of the northeastern United States. In Europe, winter moth can be found across the continent in locations as far east as the Ural mountains and as far north as northern Fennoscandia (Blackburn et al. 2020). Yet, in North America, this species has a primarily maritime distribution (Blackburn et al. 2020), and for some reason has not invaded the interior of the continent. Winter temperatures are frequently identified as limiting factors for insect distributions, and previously Broadley (2018) deployed winter moth egg masses at sites across the northeastern United States to determine if cold hardiness of the eggs (the overwintering life stage for this species), might explain this coastal pattern. Surprisingly, Broadley (2018) found that winter temperatures had little effect on egg mortality in the region, and survivorship was high in all but the most extreme localities. If, however, adult exposure to extreme winter events is the limiting life stage for this species, then any allochronic mismatch between the optimal timing of flight in its native compared to its invaded environment could help explain why winter moth has not become a more widespread invasive species.

Two factors that have been proposed to limit winter moth flight are subzero temperatures (O'Donnell and Groden 2017) and heavy snowfall (Peterson and Nilssen 1998). Factors that might limit Bruce spanworm flight are unknown, but we expect that given their shared evolutionary histories, that they might be the same as for winter moth. Here, we found that the native Bruce spanworm consistently completes its mating flights prior to the onset of subzero temperatures and heavy snow accumulations (Supplementary Figs. S3 and S4). In contrast, in both Massachusetts and Connecticut, winter moth flight continued and then appeared to be prematurely terminated by subzero temperatures (both transects in 2017), and heavy snow fall (Massachusetts transect in 2018). Interestingly, in 2018 temperatures

remained above 0 °C along the Connecticut transect and little snow was recorded that year. While Bruce spanworm flight occurred and terminated at its usually time that year, the flight of winter moth continued in Connecticut through calendar day 364, and likely continued much longer after that, but we were unable to collect moths between 22 Nov 2018 and 15 Jan 2019 as a result of a United States Government shutdown and furlough event that prevented Forest Service employees from collecting traps during that period.

In its native range, a recent study found that winter moth flight times were highly consistent over a 10-yr period (Salis et al. 2018), and thus it is likely that late-season exposure will continue to be a major source of mortality or will result in reduced fitness for winter moth individuals due to lost mating opportunities during subzero or dense snow-pack days until this species adapts to the North American environment in regards to the allochronic timing of its mating flights. However, any shift to earlier seasonal flight will result in more mating opportunities with Bruce spanworm. As a result, a new stable allochronic equilibrium will need to develop that balances the selective pressures of reinforcement to avoid hybridization with Bruce spanworm on one end with completing their mating flights prior to the occurrence of extreme winter events on the other. As such, we believe this system presents many exciting opportunities to study the genomic and evolutionary consequences of hybridization between an invasive and native species on a temporal time scale.

Acknowledgments

The authors would like to thank David Mikus and DeAdra Newman for their field and laboratory assistance, Ron Weeks at the USDA APHIS for his support of research into the evolution of winter moth populations in North America, and Michelle Labbé, Rodger Gwiazdowski, and 3 anonymous reviewers for their comments on earlier versions of this manuscript.

Funding

Funding was provided by grants from USDA APHIS AP17PPQS&T00C068, AP18PPQS&T00C070, and AP19PPQ FO000C125 awarded to J.S.E.

Author Contributions

Jeremy Andersen (Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Investigation-Equal, Methodology-Equal, Writing – original draft-Equal, Writing – review & editing-Equal), Nathan Havill (Conceptualization-Equal, Formal analysis-Equal, Investigation-Equal, Methodology-Equal, Resources-Equal, Writing – original draft-Equal, Writing – review & editing-Equal), Jennifer Chandler (Data curation-Equal, Formal analysis-Equal, Writing – original draft-Equal, Writing – review & editing-Equal), George Boettner (Data curation-Equal, Investigation-Equal, Writing – original draft-Equal, Writing – review & editing-Equal), Brian Griffin (Data curation-Equal, Methodology-Equal, Writing – original draft-Equal, Writing – review & editing-Equal), Joseph S. Elkinton (Conceptualization-Equal, Funding acquisition-Equal, Investigation-Equal, Methodology-Equal, Project administration-Equal, Resources-Equal, Supervision-Equal, Writing – original draft-Equal, Writing – review & editing-Equal)

Data Availability

All data has been provided in the Supplemental Appendices.

Supplementary Material

Supplementary material is available at *Environmental Entomology* online.

References

Allendorf FW, Leary RF, Spruell P, Wenburg JK. The problems with hybrids: setting conservation guidelines. *Trends Ecol Evol*. **2001**:16:613–622.

Alpert P. The advantages and disadvantages of being introduced. *Biol Invasions*. **2006**:8(7):1523–1534. https://doi.org/10.1007/s10530-005-5844-z

Andersen JC, Havill NP, Boettner GH, Chandler JL, Caccone A, Elkinton JS. Real-time geographic settling of a hybrid zone between the invasive winter moth (*Operophtera brumata* L.) and the native Bruce spanworm (*O. bruceata* Hulst). *Mol Ecol*. **>2022**:31:>6617–6633.

Andersen JC, Havill NP, Broadley HJ, Boettner GH, Caccone A, Elkinton JS. Widespread hybridization among native and invasive species of *Operophtera* moths (Lepidoptera: Geometridae) in Europe and North America. *Biol Invasions*. **2019**:21(11):3383–3394. https://doi.org/10.1007/s10530-019-02054-1

Anderson EC. Bayesian inference of species hybrids using multilocus dominant genetic markers. *Philos Trans R Soc B Biol Sci*. **2008**:363(1505):2841–2850. https://doi.org/10.1098/rstb.2008.0043.

Anderson EC, Thompson EA. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*. **2002**:160(3):1217–1229. https://doi.org/10.1093/genetics/160.3.1217.

Blackburn LM, Elkinton JS, Havill NP, Broadley HJ, Andersen JC, Liebhold AM. Predicting the invasion range for a highly polyphagous and widespread forest herbivore. *Neobiota*. **2020**:59:1–20.

Broadley HJ. Impact of native natural enemies on populations of the invasive winter moth (*Operophtera brumata* L.) in the northeast United States [*PhD*]. University of Massachusetts Amherst; **2018**.

Brockerhoff EG, Liebhold AM, Jactel H. The ecology of forest insect invasions and advances in their management. *Can J For Res*. **2006**:36(2):263–268. https://doi.org/10.1139/x06-013.

Butlin R. Speciation by reinforcement. *Trends Ecol Evol*. **1987**:2(1):8–13. https://doi.org/10.1016/0169-5347(87)90193-5

Cardé RT, Baker TC. Sexual Communication with Pheromones. In: Bell WJ, Cardé RT, editors. *Chemical ecology of insects*. New York (NY): Chapman and Hall; **1984**. p. 355–383.

Cordeiro EMG, Pantoja-Gomez LM, de Paiva JB, Nascimento A, Omoto C, Michel AP, Correa AS. Hybridization and introgression between *Helicoverpa armigera* and *H. zea*: an adaptational bridge. *BMC Evol Biol*. **2020**:20:>61.

Correa AS, Cordeiro EMG, Omoto C. Agricultural insect hybridization and implications for pest management. *Pest Manag Sci*. **2019**:75:2857–2864.

Diaz-Puente B, Pita A, Uribe J, Cuellar-Pinzon J, Guinez R, Presa P. A biogeography-based management for *Mytilus chilensis*: the genetic hodgepodge of Los Lagos versus the pristine hybrid zone of the Magellanic ecotone. *Aquat Conserv Mar Freshwater Ecosyst*. **2020**:30:412–425.

Dobzhansky T. Speciation as a stage in evolutionary divergence. *Am Nat*. **1940**:74:312–321.

Ejsmont-Karabin J, Hutorowicz A, Kapusta A, Stawecki K, Tunowski J, Zdanowski B. Rotifers in heated Konin lakes-a review of long-term observations. *Water*. **2020**:12(6):1660. https://doi.org/10.3390/w12061660.

Elkinton J, Boettener G, Liebhold A, Gwiazdowski R. Biology, spread, and biological control of winter moth in the Eastern United States. In: Reardon R, editor. *USDA forest service publication*. >Morgantown (WV): USDA Forest Service; **2015**. p. 22.

Elkinton JS, Boettner GH, Broadley HJ. Successful biological control of winter moth, *Operophtera brumata*, in the northeastern United States. *Ecol Appl*. **2021**:31(5):e02326.

Elkinton JS, Boettner GH, Sremac M, Gwiazdowski R, Hunkins RR, Callahan J, Scheufele SB, Donahue CP, Porter AH, Khrimian A, *et al*. Survey for winter moth (Lepidoptera: Geometridae) in northeastern North America with pheromone-baited traps and hybridization with the native Bruce spanworm (Lepidoptera: Geometridae). *Ann Entomol Soc Am*. **2010**:103(2):135–145. https://doi.org/10.1603/an09118

Elkinton JS, Lance D, Boettner G, Khrimian A, Leva N. Evaluation of pheromone-baited traps for winter moth and Bruce spanworm (Lepidoptera: Geometridae). *J Econ Entomol*. **2011**:104(2):494–500. https://doi.org/10.1603/ec09322

Elkinton JS, Liebhold A, Boettner GH, Sremac M. Invasion spread of *Operophtera brumata* in northeastern United States and hybridization with *O-bruceata*. *Biol Invasions*. **2014**:16(11):2263–2272. https://doi.org/10.1007/s10530-014-0662-9

Fergus DJ, deCarvalho TN, Shaw KL. Genetically regulated temporal variation of novel courtship elements in the Hawaiian cricket genus *Laupala*. *Behav Genet*. **2011**:41(4):607–614. https://doi.org/10.1007/s10519-010-9397-2

Fischer MJ, Havill NP, Brewster CC, Davis GA, Salom SM, Kok LT. Field assessment of hybridization between *Laricobius nigrinus* and *L. rubidus*, predators of Adelgidae. *Biol Control*. **2015**:82:1–6. https://doi.org/10.1016/j.biocontrol.2014.12.002

Fisher R. *The Genetical theory of natural selection*. Oxford (UK): The Clarendon Press; **1930**.

Gallien L, Carboni M. The community ecology of invasive species: where are we and what's next? *Ecography*. **2017**:40:335–352.

Gay L, Crochet PA, Bell DA, Lenormand T. Comparing clines on molecular and phenotypic traits in hybrid zones: a window on tension zone models. *Evolution*. **2008**:62(11):2789–2806. https://doi.org/10.1111/j.1558-5646.2008.00491.x

Griffin BP, Chandler JL, Andersen JC, Havill NP, Elkinton JS. The reliability of genitalia morphology to monitor the spread of the invasive winter moth (Lepidoptera: Geometridae) in eastern North America. *Environ Entomol*. **2020**:49(6):1492–1498. https://doi.org/10.1093/ee/nvaa122

Groh JS, Cronk QCB. Phenotypic evidence for an extensive mosaic hybrid zone between two species of columbine, *Aquilegia flavescens* and *A. formosa*. *Botany*. **2020**:98:459–467.

Harrison RG, Larson EL. Hybridization, introgression, and the nature of species boundaries. *J Hered*. **2014**:105(S1):795–809. https://doi.org/10.1093/jhered/esu033

Havill NP, Davis G, Mausel DL, Klein J, McDonald R, Jones C, Fischer M, Salom S, Caccone A. Hybridization between a native and introduced predator of Adelgidae: an unintended result of classical biological control. *Biol Control*. **2012**:63(3):359–369. https://doi.org/10.1016/j.biocontrol.2012.08.001

Havill NP, Elkinton J, Andersen JC, Hagen SB, Broadley HJ, Boettner GJ, Caccone A. Asymmetric hybridization between non-native winter moth, *Operophtera brumata* (Lepidoptera: Geometridae), and native Bruce spanworm, *Operophtera bruceata*, in the Northeastern United States, assessed with novel microsatellites and SNPs. *Bull Entomol Res*. **2017**:107(2):241–250. https://doi.org/10.1017/S0007485316000857

Herder F, Schliewen UK, Geiger MF, Hadiaty RK, Gray SM, McKinnon JS, Walter RP, Pfaender J. Alien invasion in Wallace's Dreamponds: records of the hybridogenic "flowerhorn" cichlid in Lake Matano, with an annotated checklist of fish species introduced to the Malili Lakes system in Sulawesi. *Aquat Invasions*. **2012**:7:521–535.

Hinton WF. Natural hybridization and extinction of a population of *Physalis virginiana* (Solanacea). *Am J Bot*. **1975**:62(2):198–202. https://doi.org/10.1002/j.1537-2197.1975.tb14053.x

Irimia RE, Hierro JL, Branco S, Sotes G, Cavieres LA, Eren O, Lortie CJ, French K, Callaway RM, Montesinos D. Experimental admixture among geographically disjunct populations of an invasive plant yields a global mosaic of reproductive incompatibility and heterosis. *J Ecol*. **2021**:109(5):2152–2162. https://doi.org/10.1111/1365-2745.13628

Jones EI, Gomulkiewicz R. Biotic interactions, rapid evolution, and the establishment of introduced species. *Am Nat*. **2012**:179(2):E28–E36. https://doi.org/10.1086/663678

Levin DA, FranciscoOrtega J, Jansen RK. Hybridization and the extinction of rare plant species. *Conserv Biol*. **1996**:10(1):10–16. https://doi.org/10.1046/j.1523-1739.1996.10010010.x

Levine JM, Adler PB, Yelenik SG. A meta-analysis of biotic resistance to exotic plant invasions. *Ecol Lett*. **2004**:7(10):975–989. https://doi.org/10.1111/j.1461-0248.2004.00657.x

Liou LW, Price TD. Speciation by reinforcement of premating isolation. *Evolution*. **1994**:48(5):1451–1459. https://doi.org/10.1111/j.1558-5646.1994.tb02187.x

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol Appl*. **2000**:10:689–710.

Murrell C, Gerber E, Krebs C, Parepa M, Schaffner U, Bossdorf O. Invasive knotweed affects native plants through allelopathy. *Am J Bot*. **2011**:98(1):38–43. https://doi.org/10.3732/ajb.1000135

O'Brien PP, Bowman J, Coombs AB, Newar SL, Garroway CJ. Winter nest trees of sympatric northern (*Glaucomys sabrinus*) and southern (*Glaucomys volans*) flying squirrels: a test of reinforcement in a hybrid zone. *Can J Zool*. **2021**:99(10):859–866. https://doi.org/10.1139/cjz-2021-0086

O'Donnell K, Groden E. Variation in captures of adult winter moths (*Operophtera brumata*) in coastal Maine over two years. *Northeast Nat*. **2017**:24(sp7):B72–B80. https://doi.org/10.1656/045.024.s709

Paillex A, Castella E, zu Ermgassen P, Gallardo B, Aldridge DC. Large river floodplain as a natural laboratory: non-native macroinvertebrates benefit from elevated temperatures. *Ecosphere*. **2017**:8(10):e01972. https://doi.org/10.1002/ecs2.1972

Peterson NA, Nilssen AC. Late autumn eclosion in the winter moth *Operophtera brumata*: compromise of selective forces in life-cycle timing. *Ecol Entomol*. **1998**:23(4):417–426. https://doi.org/10.1046/j.1365-2311.1998.00155.x

Pfennig KS, Kelly AL, Pierce AA. Hybridization as a facilitator of species range expansion. *Proc R Soc B Biol Sci*. **2016**:283:20161329.

Pfennig KS, Simovich MA. Differential selection to avoid hybridization in two toad species. *Evolution*. **2002**:56(9):1840–1848. https://doi.org/10.1111/j.0014-3820.2002.tb00198.x

Popovic I, Bierne N, Gaiti F, Tanurdzic M, Riginos C. Pre-introduction introgression contributes to parallel differentiation and contrasting hybridization outcomes between invasive and native marine mussels. *J Evol Biol*. **2021**:34(1):175–192. https://doi.org/10.1111/jeb.13746

Prentis PJ, White EM, Radford IJ, Lowe AJ, Clarke AR. Can hybridization cause local extinction: a case for demographic swamping of the Australian native *Senecio pinnatifolius* by the invasive *Senecio madagascariensis*? *New Phytol*. **2007**:176(4):902–912. https://doi.org/10.1111/j.1469-8137.2007.02217.x

Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ. Adaptive evolution in invasive species. *Trends Plant Sci*. **2008**:13:288–294.

Punzalan D, Fang JT, Chen W, Rowe L. Divergence in life history and behaviour between hybridizing *Phymata* ambush bugs (Heteroptera: Reduviidae). *Biol J Linn Soc*. **2021**:133(3):796–805. https://doi.org/10.1093/biolinnean/blab006

R Core Team. *R: a language and environment for statistical computing computer program*. Vienna (Austria): R Foundation for Statistical Computing; **2022**.

Rhymer JM, Simberloff D. Extinction by hybridization and introgression. *Annu Rev Ecol Syst*. **1996**:27(1):83–109. https://doi.org/10.1146/annurev.ecolsys.27.1.83

Salis L, van den Hoorn E, Beersma DGM, Hut RA, Visser ME. Photoperiodic cues regulate phenological carry-over effects in an herbivorous insect. *Funct Ecol*. **2018**:32:171–180.

Santos H, Rousselet J, Magnoux E, Paiva MR, Branco M, Kerdelhue C. Genetic isolation through time: allochronic differentiation of a phenologically atypical population of the pine processionary moth. *Proc R Soc B Biol Sci*. **2007**:274:935–941.

Schulz AN, Lucardi RD, Marsico TD. Strengthening the ties that bind: an evaluation of cross-disciplinary communication between invasion ecologists and biological control researchers in entomology. *Ann Entomol Soc Am*. **2021**:114(2):163–174. https://doi.org/10.1093/aesa/saaa052

Scriber JM, Ording GJ. Ecological speciation without host plant specialization; possible origins of a recently described cryptic *Papilio* species. *Entomol Exp Appl*. **2005**:115(1):247–263. https://doi.org/10.1111/j.1570-7458.2005.00285.x

Servedio MR, Noor MAF. The role of reinforcement in speciation: Theory and data. *Annu Rev Ecol Evol Syst*. **2003**:34:339–364.

Sirkia PM, McFarlane SE, Jones W, Wheatcroft D, Alund M, Rybinski J, Qvarnstrom A. Climate-driven build-up of temporal isolation within a recently formed avian hybrid zone. *Evolution*. **2018**:72(2):363–374. https://doi.org/10.1111/evo.13404

Spencer HG, McArdle BH, Lambert DM. A theoretical investigation of speciation by reinforcement. *Am Nat*. **1986**:128(2):241–262. https://doi.org/10.1086/284557

Teixeira MC, Turchetto C, Schnitzler CK, Callegari-Jacques SM, Freitas LB. Could the reproductive system explain the stability and long-term persistence in a natural hybrid zone of *Petunia* (Solanaceae)? *Acta Bot Bras*. **2021**:35(4):660–669. https://doi.org/10.1590/0102-33062020abb0514

Thornton MM, Shrestha R, Wei Y, Thornton PE, Kao S, Wilson BE. *Daymet: daily surface weather data on a 1-km grid for North America; version 4*. Oak Ridge (TN): ORNL DAAC; **2020**.

Todesco M, Pascual MA, Owens GL, Ostevik KL, Moyers BT, Hubner S, Heredia SM, Hahn MA, Caseys C, Bock DG, *et al*. Hybridization and extinction. *Evol Appl*. **2016**:9(7):892–908. https://doi.org/10.1111/eva.12367

Troubridge JT, Fitzpatrick SM. A revision of the North-American *Operophtera* (Lepidoptera, Geometridae). *Can Entomol*. **1993**:125(2):379–397. https://doi.org/10.4039/ent125379-2

Valencia-Montoya WA, Elfekih S, North HL, Meier JI, Warren IA, Tay WT, Gordon KHJ, Specht A, Paula-Moraes SV, Rane R, *et al*. Adaptive introgression across semipermeable species boundaries between local *Helicoverpa zea* and invasive *Helicoverpa armigera* moths. *Mol Biol Evol*. **2020**:37(9):2568–2583. https://doi.org/10.1093/molbev/msaa108

Ward SM, Gaskin JF, Wilson LM. Ecological genetics of plant invasion: what do we know? *Invasive Plant Sci Manage*. **2008**:1(1):98–109. https://doi.org/10.1614/ipsm-07-022.1

Wolf DE, Takebayashi N, Rieseberg LH. Predicting the risk of extinction through hybridization. *Conserv Biol*. **2001**:15(4):1039–1053. https://doi.org/10.1046/j.1523-1739.2001.0150041039.x

Zemanova MA, Knop E, Heckel G. Introgressive replacement of natives by invading Arion pest slugs. *Sci Rep*. **2017**:7(1):>14908.