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Population dynamics of the Lyme disease bacterium, *Borrelia burgdorferi*, during rapid range expansion in New York State

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Abstract

Recent changes in climate and human land-use have resulted in alterations of the geographic range of many species, including human pathogens. Geographic range expansion and population growth of human pathogens increase human disease risk. Relatively little empirical work has investigated the impact of range changes on within-population variability, a contributor to both colonization success and adaptive potential, during the precise time in which populations are colonized. This is likely due to the difficulties of collecting appropriate natural samples during the dynamic phase of migration and colonization. We systematically collected blacklegged ticks (*Ixodes scapularis*) across New York State (NY), USA, between 2006 and 2019, a time period coinciding with a rapid range expansion of ticks and their associated pathogens including *Borrelia burgdorferi*, the etiological agent of Lyme disease. These samples provide a unique opportunity to investigate the population dynamics of human pathogens as they expand into novel territory. We observed that founder effects were short-lived, as gene flow from long-established populations

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Author contributions

ZO and DB organized and designed the study. MP, RF, VV, CO, JH, and PB organized and performed the fieldwork and subsequent DNA extractions. ZO performed amplifications and PP and SR developed and performed LOG assay. ZO analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare no conflicts of interest.

Benefit-sharing statement

Benefits generated: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

brought almost all *B. burgdorferi* lineages to newly colonized populations within just a few years of colonization. By seven years post-colonization, *B. burgdorferi* lineage frequency distributions were indistinguishable from long-established sites, indicating that local *B. burgdorferi* populations experience similar selective pressures despite geographic separation. The *B. burgdorferi* lineage dynamics elucidate the processes underlying the range expansion and demonstrate that migration into, and selection within, newly colonized sites operate on different time scales.

Keywords

range expansion; *Borrelia burgdorferi* ; dispersal; population dynamics

Introduction

Geographic range changes of wildlife are accelerating as a result of climate change (Chen et al., 2011; Lenoir et al., 2020; Walther et al., 2009), human land use alterations (Calinger et al., 2015; Lee et al., 2017; With, 2002), and globalized trade and transportation networks (Banks et al., 2015; Hulme, 2009; Seebens et al., 2013, 2017). Range expansions can cause fundamental and irreversible changes to ecological communities that can precipitate the emergence of human infectious diseases (Doherty et al., 2016; Dukes & Mooney, 2004; Halliday et al., 2020; Keesing et al., 2010). Similarly, the expanding range of infectious disease systems can have dramatic impacts on natural ecology, public health, and economics (Crowl et al., 2008; Mack et al., 2000; Morens et al., 2004). Despite the theoretical consequences of genetic diversity on ecological and evolutionary factors such as colonization success and adaptive potential, relatively little empirical work has investigated the genetic diversity of pathogens as they invade novel areas. Here, we investigate the population dynamics of the causative agent of Lyme disease, *Borrelia burgdorferi*, using samples systematically collected across New York State (NY) during a timeframe when the geographic range of this disease system expanded significantly (Khatchikian et al., 2015; Prusinski et al., 2014; Tran et al., 2021, 2022).

Lyme disease is the most common vector-borne zoonotic disease of humans in the United States (Schwartz et al., 2017). The composition of local *B. burgdorferi* lineages affects the risk of human infection, as different lineages vary in their ability to infect humans (Brisson et al., 2011; Dykhuizen et al., 2008; Hanincová et al., 2013; Wormser et al., 2008). Specifically, a small subset of lineages (A, B, I, and K) are responsible for the majority of disseminating infections in humans (Seinost et al., 1999; Dykhuizen et al., 2008; Wormser et al., 2008). Therefore, identifying the lineage composition in newly colonized *B. burgdorferi* populations should allow for more accurate assessments of public health risks in newly exposed human populations. In areas with decades-old populations, most *B. burgdorferi* lineages are present at relatively even frequencies due to balancing selection (Brisson & Dykhuizen, 2004; Qiu et al., 2002; Wang et al., 1999), although migration of each lineage was necessary to seed the initial population. The recent geographic range expansion of the Lyme disease system in the northeastern United States provides a natural experiment to investigate both the rate and order in which *B. burgdorferi* lineages arrive in newly colonized

areas as well as the subsequent lineage frequency dynamics caused by further migration or natural selection.

Population genetics models of range expansion predict that different dispersal patterns should have qualitatively different impacts on diversity in invaded areas (Bialozyt et al., 2006; Hewitt, 1999; Ibrahim et al., 1996). For example, slow range expansions are expected to retain most of the diversity contained in the source population (often called travelling waves), resulting in little population genetic structure across the geographic range (Arenas et al., 2012; Garnier & Lewis, 2016; Roques et al., 2012). By contrast, long-distance dispersal often results in isolated populations founded by a small number of individuals that represent a subset of the diversity in the source population leading to significant population genetic structure (Bialozyt et al., 2006; Ibrahim et al., 1996; Ogden et al., 2013). While empirical data broadly support these theoretical predictions, nearly all data are derived from surveys conducted years or decades after the migration and colonization process occurred (Dlugosch & Parker, 2008; Hewitt, 1999). Data collected during a range expansion are necessary to identify likely dispersal patterns and to evaluate the impact of migration on diversity in invaded areas.

By characterizing the impacts of the recent range expansion on the spatial and temporal dynamics of *B. burgdorferi* diversity, we can identify the evolutionary and ecological mechanisms underlying the spread of these pathogens. We systematically collected blacklegged ticks (*Ixodes scapularis*) and their associated pathogens across NY between 2003 and 2019, a location and timeframe in which the Lyme disease system experienced a substantial geographic range expansion (Khatchikian et al., 2015; Prusinski et al., 2014; Tran et al., 2021, 2022). We determined the colonization dynamics of *B. burgdorferi* lineages in newly colonized locations. Further, we characterized temporal changes in *B. burgdorferi* lineage frequencies in invaded areas up to ten years after the populations were first colonized. The conclusions from these studies are keenly relevant to public health as climate and land use changes continue to spur unprecedented movement of vectors and pathogens into contact with previously unexposed human populations.

Materials and Methods

Tick collection.

Host-seeking *I. scapularis* ticks were systematically collected as part of routine tick-borne pathogen surveillance by the New York State Department of Health between 2003–2019 (Prusinski et al., 2014). Tick collection data are available at (https://doi.org/10.17632/7xgr36j9k5.2). Briefly, ticks were collected from publicly accessible lands based on any one of the following criteria: northern hardwood forest type; suitable habitat for *Ixodes scapularis*, white-tailed deer (*Odocoileus virginianus*) and *Peromyscus* species mice; potential presence of *I. scapularis* as indicated by NYSDOH passive surveillance; potential for human exposure to ticks; or epidemiological links to locally acquired tick-borne disease cases. Most sites were located on state or county-owned land such as wildlife management areas, nature preserves and parks. Collection sites were visited during periods of peak adult *I. scapularis* activity (October-November). Some sites were sampled on an annual basis, while others were sampled on a rotational basis every 2 to 5 years. Ticks were collected by

standardized dragging, flagging, and walking surveys using 1 m^2 of white flannel or canvas and at least 1000 m^2 were sampled during each collection event regardless of whether ticks were encountered. Collected ticks were stored in 80–100 % ethanol at 4 °C until sorted by developmental stage and confirmed to be *I. scapularis* using a compound microscope and dichotomous keys (Durden & Keirans, 1996; Keirans & Clifford, 1978). Ticks were then stored in sterilized 1.5 mL Snap-Cap Microcentrifuge Flex-Tubes™ (item 022364111, Eppendorf North America, Inc., Hauppauge, NY) and preserved in 80–100 % ethanol at −20 °C until nucleic acid extraction.

Whole tick DNA extraction and tick-borne pathogen screening.

Prior to 2013, whole tick DNA extraction was performed as described in Prusinski et al., 2014, after which partial automation of the extraction process by way of the QIAcube HT robotic platform (Qiagen, Valencia, CA) was performed as described in Piedmonte et al., 2018. Briefly, individual ticks were double rinsed with nuclease-free distilled water and placed in 2.0-mL round-bottomed Safe-Lock tubes (Eppendorf, North America, Inc., Hauppauge, NY) with two 4-mm stainless steel grinding beads and 205 uL PBS, pH 7.2. Samples were ground using an electric tissue homogenizer (MM300, Retch, USA) and centrifuged at 13,000 rpm for 2 min, with 200 uL of the resulting supernatant subjected to total gDNA extraction. Prior to 2013, tick and pathogen gDNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) following the manufacturer's recommendations for supplementary protocol: "Purification of Total DNA from Insects." From 2013 onward, tick and pathogen gDNA was extracted and purified using the automated QIAcube HT system (Qiagen, Valencia, CA) and QIAamp 96 DNA QIAcube HT Kit, following the corresponding kit protocol automated for the QIAcube HT robotic platform. Both methods employ silica-based columns to extract and purify DNA from tissue samples, with the latter being in a 96-well format and using automated pipette and vacuum steps and the former using individual spin columns, manual pipetting, and centrifugation to purify and elute samples. DNA concentrations and yield were comparable with and without automation, as determined by two-sample, two-tailed t-test of DNA concentration values (mean ± SE) for 40 tick samples prepared using the manual (mean 3.34 ± 0.22 ng/uL) and automated (mean 3.58 ± 0.32 ng/uL) methods measured by NanoDrop™ 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc.) of 80 gDNA samples in total ($t = -0.65$; df = 61; $p = 0.515$). Whole tick DNA extraction was conducted in a class 2 biological safety cabinet with laminar airflow (SterilGARD III Advance, Baker Co., Sanford, ME) designated exclusively for DNA extraction, and sterile aerosol-barrier tips were used during all procedures to prevent cross contamination. DNA from individual ticks was eluted with 200 uL ultra-pure water and stored at −20 °C until polymerase chain reaction (PCR) testing. Whole tick DNA extracts were screened for *B. burgdorferi* as described in Prusinski et al., 2014. The number of ticks screened for pathogens at each site-year varied due to variation in number of ticks collected (Table S1).

Site selection and classification.

In order to track *B. burgdorferi* population dynamics at fine temporal scale, we selected a subset of collection sites that satisfied the following criteria: (1) annual sampling occurred over a minimum of five consecutive years, and (2) at least 15 *B. burgdorferi*-positive

(*Bb*+) ticks were collected each year (Table S1). Eight sites satisfying these criteria were chosen to maximize spatial distribution across NY (Table S2). Three of the eight sites (Cattaraugus, Erie, and Monroe) contained newly colonized *B. burgdorferi* populations. Prior to *B. burgdorferi* colonization at each of these sites, *I. scapularis* ticks were collected but few or none were *B. burgdorferi*-positive. The year of colonization was labeled as "Year 1" and was the first year in which 15 *Bb*+ ticks were collected at each of these three sites. Five sites (Delaware, Orange, Putnam, Saratoga, and Sullivan) supported established *B. burgdorferi* populations. These sites all had high *B. burgdorferi* prevalence in collected ticks (>40 % positive for the presence of *B. burgdorferi*), either in the first year they were sampled or in years prior to the first year included in this study. Additionally, these sites are all within the historically endemic range of Lyme disease in NY. "Year 1" at these five sites is the first year for which the inclusion criteria were met (that is, five consecutive sampling years with 15 *Bb*+ ticks collected).

Sample selection and lineage typing (*ospC*).

For each year that a site was included in the study, we randomly selected 15 *Bb*+ ticks per site-year to undergo lineage typing by way of *ospC* genotyping (Pearson et al., 2022). A nested PCR protocol was used to amplify the *ospC* gene (Devevey et al., 2015), a highly variable genetic locus found to be in near complete linkage disequilibrium with chromosomal loci in isolates from the Northeastern United States (Bunikis et al., 2004; Attie et al., 2007; Hanincová et al., 2008) and commonly used to identify *B. burgdorferi* lineages (Brisson & Dykhuizen, 2004; Pearson et al., 2022; Qiu et al., 2002; Wang et al., 1999). Amplification success was determined by visualization of a band on a 1 % ethidium bromide agarose gel and concentration was quantified using the Qubit dsDNA HS Assay Kit (Invitrogen). Samples were genotyped with the Luminex *ospC* genotyping (LOG) assay as described in Pearson et al., 2022. Briefly, *ospC* amplicons were incubated with allele-specific primers and biotinylated nucleotides were incorporated during an extension step. Biotinylated products were hybridized to spectrally distinct microspheres in an *ospC* allele-specific manner. The samples were analyzed on a Luminex MAGPIX instrument which detects fluorescent signals corresponding to the presence of 19 different *ospC* genotypes. The number of DNA extracts from which the *ospC* gene was successfully amplified and genotyped for each site-year is reported in Table S3.

Statistical analysis.

All statistical analyses were conducted in R v. 4.3.1 (R Core Team, 2023). Annual differences in lineage richness (number of unique lineages) and lineage diversity (Simpson's diversity index) between newly colonized and established populations were statistically investigated using t-tests. Lineage frequency distributions were aggregated over three-year periods within each site to reduce stochastic variability due to small sample sizes when comparing populations. Population genetic differentiation among lineage frequency distributions was assessed using pairwise *Fst* values, calculated using the *pairwise.WCfst* function in the hierfstat package (Goudet & Jombart, 2022). Statistical significance in population genetic differentiation among newly colonized sites, among established populations, and between newly colonized and established populations was assessed using permutation tests with 100,000 iterations. Temporal stability of lineage distributions was

investigated using pairwise *Fst* values between consecutive time points with statistical significance determined with permutation tests (100,000 iterations). Pearson's correlation coefficient was used to assess the association between lineage prevalence within established populations and lineage prevalence in newly colonized populations during the first year of colonization. *B. burgdorferi* lineages were binned into "low" (<7 %; 7 lineages), "medium" (7–14 %; 7 lineages), and "high" (>14 %; 5 lineages) prevalence groups based on prevalence in established populations across all years sampled. Statistical significance of annual differences in prevalence of the most common lineage was assessed with permutation tests (100,000 iterations).

Results

Lineage richness and diversity in local populations

We identified 19 unique *B. burgdorferi* lineages among the 1,076 *Bb*+ adult *I. scapularis* ticks collected from 8 sites over 14 years (Fig. 1). Infected ticks were infected with an average of 1.87 *B. burdorferi* lineages. The number of unique *B. burgdorferi* lineages at a site (lineage richness) and the Simpson's diversity index (lineage diversity) were significantly lower in the first two years at newly colonized *B. burgdorferi* populations than in established populations (Fig. 2). Established populations had high lineage richness, containing an average of 11.4 (10–13) unique *B. burgdorferi* lineages per site in the first sampling year. In contrast, newly colonized populations had significantly lower lineage richness with only 4–8 unique *B. burgdorferi* lineages per site in the first year post-colonization (p=0.027). Similarly, established populations had significantly higher lineage diversity than newly colonized populations (p=0.045). That is, pairs of *B. burgdorferi* lineages randomly selected from newly colonized populations were more likely to be the same lineage than pairs of randomly selected *B. burgdorferi* lineages from established populations.

Lineage richness and diversity in established sites remained stable across sampling years (Fig. 2). In newly colonized populations, by contrast, there was a marked increase in lineage richness over the first three years of sampling, after which the number of unique *B. burgdorferi* lineages remained stable and at magnitudes similar to established sites (Fig. 2a). Similarly, lineage diversity increased in newly colonized populations during the first three years after establishment to levels similar to the diversity measured in established sites (Fig. 2b). Lineage richness and diversity metrics were statistically indistinguishable between established populations and populations that had been colonized for at least four years.

Differentiation among populations

Despite sharing similar lineage richness and diversity temporal profiles (Fig. S1), newly colonized populations were distinct from one another and from established populations, determined by comparing their lineage frequency distributions (Fig. 3). Established populations remained similar to one another throughout the sampling period (Fst ~ 0.013). By comparison, newly colonized populations were significantly more differentiated from each other (years 1–3 post-colonization; Fst ~ 0.060, p=0.002) and from established populations (years 1–3 post-colonization; Fst ~ 0.051, p<0.001). Recently colonized

populations became increasingly similar over time, though they still remained significantly more differentiated from each other 4–6 years after colonization (*Fst* ~ 0.030) than pairs of established populations (*Fst* ~ 0.003, p=0.011). By 7–9 years after colonization, recently colonized populations were no more differentiated from one another (*Fst* ~ 0.025) than established populations (*Fst* ~ 0.009; p=0.43).

Newly colonized populations were unstable, even after attaining similar lineage richness and diversity to established populations (Fig. 4). That is, while established populations remained similar across the sampling period (*Fst* ~ 0.002), newly colonized populations changed significantly between consecutive time points (1–3 years post-colonization vs 4–6 years post-colonization; *Fst* ~ 0.027, p=0.021). However, recently colonized populations stabilized 4–6 years post-colonization (4–6 years post-colonization vs 7–9 years post-colonization; *Fst* ~ 0.003), showing frequency distribution changes similar to established populations (*Fst* < 0.001, p=0.44).

Arrival patterns of *B. burgdorferi* lineages into new populations

The prevalence of *B. burgdorferi* lineages arriving at newly colonized populations approximates the prevalence of each lineage in established populations across all sampling years, where prevalence refers to the proportion of *Bb*+ ticks infected with a specific lineage (Fig. 5). The prevalence of a lineage in established populations is positively correlated with the prevalence of that lineage in the first year *B. burgdorferi* colonized the area (r=0.18). That is, *B. burgdorferi* lineages that were prevalent in established populations were more likely to arrive early in newly colonized populations than were lineages that were rarer in established populations.

Dominant *B. burgdorferi* lineage dynamics in newly established populations

Lineage frequency distributions were highly skewed in the first year of colonization, but quickly resembled those in established populations. The prevalence of the most common lineage in the first-year post-colonization was significantly greater than the prevalence of the most common lineage in established populations during any sampling year (70 % vs ~40 %; p<0.05) (Fig. 6). By the second-year post-colonization, the prevalence of the most common lineage had decreased significantly (p=0.036) to an average of just 42.4 %. This was statistically indistinguishable from the ~40 % prevalence of the most common lineages in established populations. The prevalence of the most common lineage remained around 40 % in all populations in all sampling years after populations had been established for at least two years.

Discussion

Borrelia burgdorferi rapidly colonizes new locations leading to the accumulation of most lineages within just three years after initial colonization. In the first two years in which new *B. burgdorferi* colonies were established, lineage composition was limited to a small subset of the total diversity found in long-established populations. However, every newly established population contained at least four *B. burgdorferi* lineages in the first year colonization was detected. Lineage diversity, a metric that incorporates lineage richness and

evenness, was also significantly lower in recently colonized populations compared to long-established populations. The limited richness and diversity in newly colonized areas resulted in frequency distributions dominated by one lineage, in stark contrast to the relatively even distributions in established sites. Lineage richness and diversity reached levels similar to those in long-established populations three years after new *B. burgdorferi* populations were colonized while the frequency distributions of lineages in newly colonized populations did not match those in established populations until years 7–9 post-colonization. That is, the composition and frequencies of *B. burgdorferi* lineages in recently colonized populations changed to become more similar to each other and to established populations over time; conversely, the lineage frequency distributions were temporally stable in established populations.

While range expansions are often associated with decreases in genetic diversity at newly colonized sites (Excoffier et al., 2009; Nei et al., 1975; Pierce et al., 2014), these losses are extremely short-lived in the recent *B. burgdorferi* range expansion. We observed founder effects in newly colonized areas with significantly lower lineage richness and diversity than in established populations ($p<0.05$). However, these founder effects eroded quickly as lineage richness and diversity in long-established populations were indistinguishable from those in recently established populations three years post-colonization, suggesting extremely rapid and ongoing migration and colonization in this system. These results resemble a moving wave front where most of the diversity from the source populations is maintained at all locations during a range expansion (Pfeifer et al., 2010; Pluess, 2011; Roques et al., 2012). Thus, migration of *B. burgdorferi* into western NY appears to be “pushed” by population growth out of source populations (Ogden et al., 2013; Roques et al., 2012). By contrast, the expected colonization pattern for species “pulled” by long-range dispersal (Bialozyt et al., 2006; Ogden et al., 2013) is for populations to be dominated by just one or two lineages for many years after initial colonization. Although these newly colonized populations are geographically distant from the long-established populations that were sampled for this study (~358 km on average), we hypothesize that the absence of major geographic barriers separating populations west of the Catskill Mountains has enabled both terrestrial vertebrates and birds to facilitate the moving wave of *B. burgdorferi* expansion.

Lineages of *B. burgdorferi* do not differ in their ability to migrate and colonize new areas. Although we expected arrival patterns of lineages to reflect their host associations (Brisson & Dykhuizen, 2004; Mechai et al., 2016; Vuong et al., 2014) – for example, bird-associated lineages would arrive before rodent-associated lineages – the prevalence of *B. burgdorferi* lineages in established populations was a better predictor of lineage migration rates. That is, the abundance of a particular *B. burgdorferi* lineage at a newly colonized population is positively associated with the relative frequency of that lineage in long-established populations. Thus, we expect that lineage prevalence in long-established populations is directly related to the number of ticks or hosts infected with that lineage arriving at new sites (Lockwood et al., 2005; Simberloff, 2009). The different subsets of *B. burgdorferi* lineages detected in the first year of colonization at each newly established site are likely due to the small differences in relative frequencies of lineages in source populations and the relatively small subset of lineages detected in newly colonized sites. That is, drawing 4–6 of 19 lineages that are all at low relative frequencies in established populations (<10.5 %) to

colonize a novel site is likely to result in only moderately overlapping subsets among newly colonized populations.

Lineage frequency distributions were significantly different among newly colonized populations, but became indistinguishable from those at long-established sites 7–9 years post-colonization. One potential mechanism that could explain these patterns is that the selective pressures shaping lineage frequency distributions are similar among geographically distant *B. burgdorferi* populations. For example, the overdispersion of lineage frequency distributions within *B. burgdorferi* populations has been shown to result from different host associations among *ospC* lineages, resulting in a form of balancing selection called multiple niche polymorphism (Brisson & Dykhuizen, 2004). Thus, the similarity in lineage frequencies among sites could suggest that vertebrate biodiversity, one of the primary forces affecting the distribution and abundance of *B. burgdorferi* lineages (Brisson & Dykhuizen, 2004; Oppler et al., 2022; Vuong et al., 2014) is relatively similar despite the geographic distance between sampled locations. Alternatively, newly colonized sites could become more similar to established sites through regular migration events from established sites into new sites that would homogenize lineage frequency distributions among sites. Additional experiments are needed to assess these explanations.

The expansion of *B. burgdorferi* populations into novel areas exposes new human populations to increased risk of acquiring Lyme disease and other tick-borne illnesses (Khatchikian et al., 2012; Tran et al., 2021). In particular, lineages A, B, I, and K, which have the greatest propensities to cause invasive infections in humans (Seinost et al., 1999; Dykhuizen et al., 2008; Wormser et al., 2008), are often among the first to arrive at newly colonized populations and continue to remain abundant throughout the colonization period. The recent geographic expansion and rapid accumulation of *B. burgdorferi* lineages at each newly colonized location could be explained by at least two potential processes, including recent increases in dispersal rates and recent increases in habitat suitability (Brederveld et al., 2011; Lehtinen & Galatowitsch, 2001; Leighton et al., 2012). Recent increases in dispersal rates could arise from recent growth of *B. burgdorferi* populations in regions closer to western NY and separated by fewer geographic barriers. For example, recent *B. burgdorferi* population growth in areas just west of the Catskill mountains may have enabled increased westward emigration. Alternatively, western locations may not have provided suitable habitat to support colonization of robust *I. scapularis* and *B. burgdorferi* populations until recently, potentially due to climate or land-use changes. Future sampling and experimental investigations can quantify the relative impact of each of these processes on geographic range expansions. Maintaining active natural surveillance programs for human pathogens remains critical as continued environmental changes could cause rapid establishment of zoonotic disease systems in proximity to additional human populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

The data and R code used in this study have been made available in the Mendeley repository (https://doi.org/10.17632/7xgr36j9k5.2).

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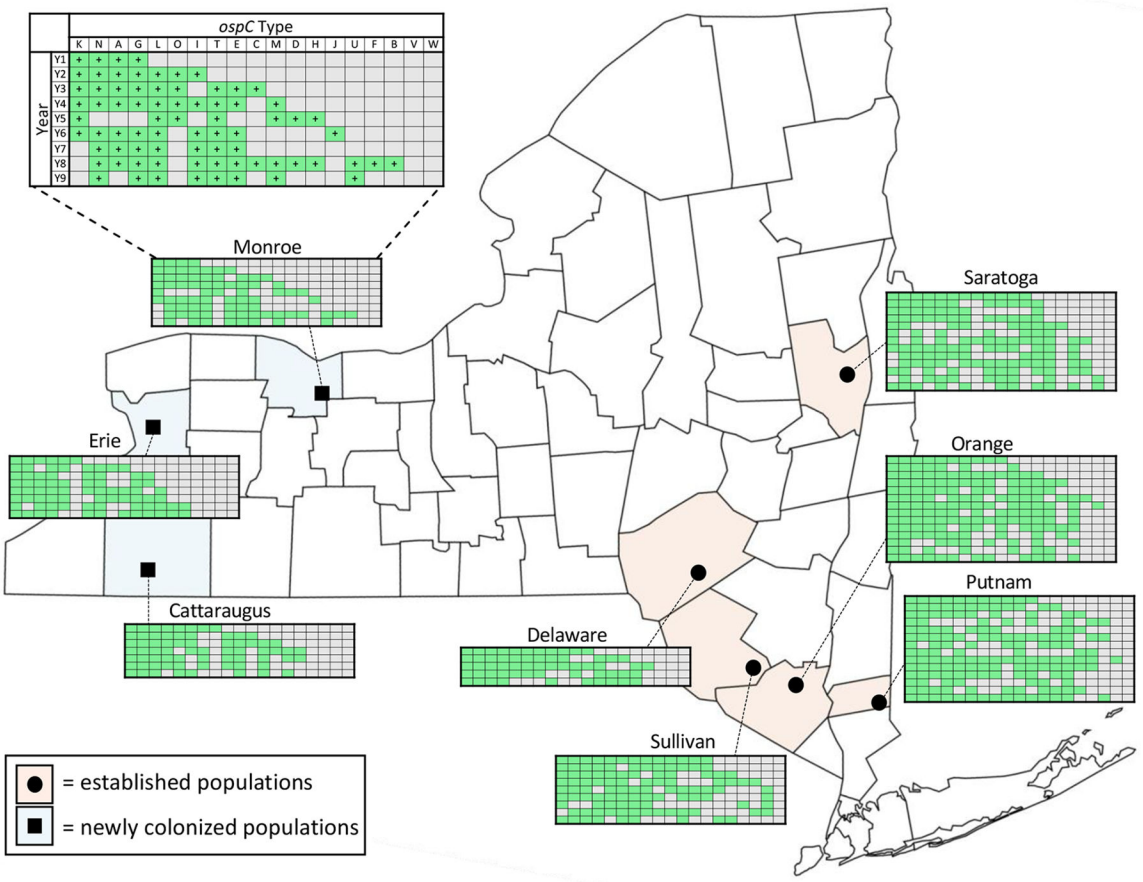

Figure 1. Sampled *B. burgdorferi* populations in New York State (NY).

B. burgdorferi in *I. scapularis* ticks were collected from eight locations across NY during a time period of range expansion. *B. burgdorferi* have been consistently abundant at five sites (red) and colonized within the sampling period at three sites (blue). Grids denote presence (green) or absence (gray) of *B. burgdorferi* lineages (columns) during each consecutively sampled year (rows) (Table S4). Order of *ospC* types differs between tables and is based on temporal order of lineage detection at each site.

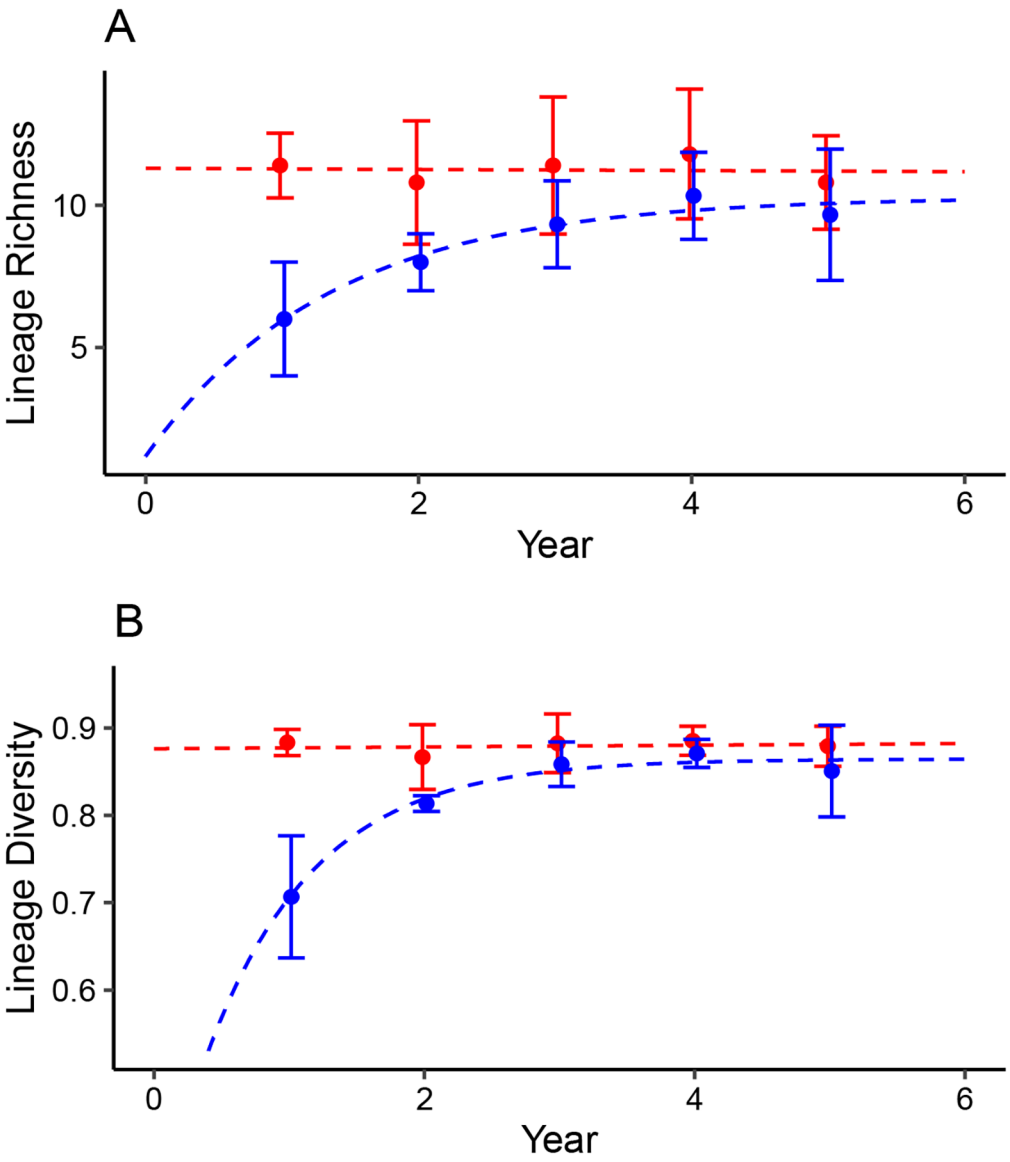

Figure 2. *B. burgdorferi* lineage richness and diversity in established populations (red points) and during the first five years post-colonization in newly colonized populations (blue points).

A. Lineage richness: The number of *B. burgdorferi* lineages is significantly higher in established populations than in populations colonized for two or fewer years ($p<0.05$). Lineage richness in areas where populations have been present for at least three years was similar to the lineage richness in established populations. B. Lineage diversity (Simpson's diversity index): Lineage diversity is significantly higher in established populations than in populations colonized for two or fewer years ($p<0.05$) after which lineage diversity was statistically indistinguishable between populations. Points are averages; error bars indicate ± 1 standard deviation. Data points for individual newly colonized populations are visualized in Figure S1.

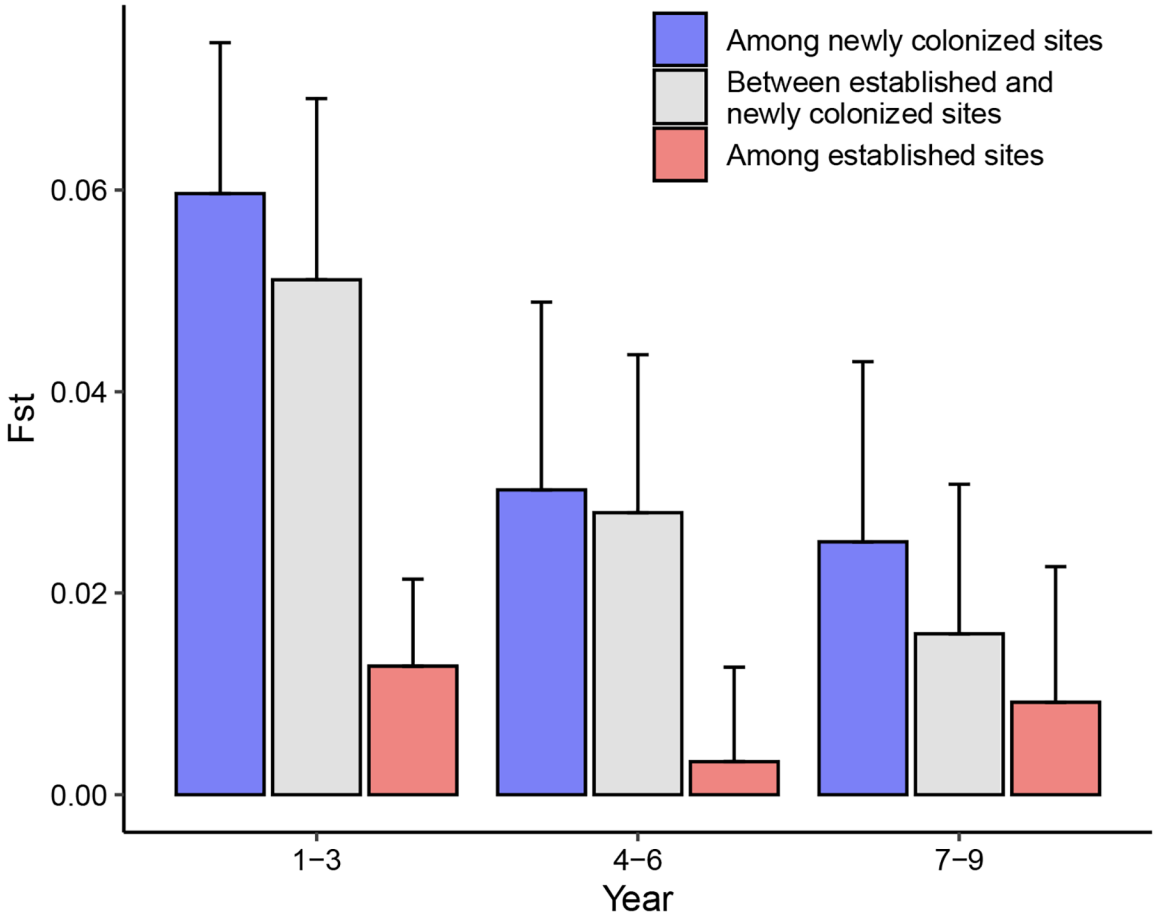

Figure 3. Population genetic differentiation (*Fst*) among and between newly colonized and established populations.

Differences among *B. burgdorferi* lineage frequency distributions were significantly greater between pairs of populations colonized within the last three years (p=0.002) and between established populations and newly colonized populations (p<0.001) than between pairs of long-established populations. Similarly, lineage frequency distributions were significantly more differentiated between pairs of populations 4–6 years post-colonization (p=0.011) or between established populations and these recently colonized populations (p<0.001) than between pairs of established populations. Bars indicate average *Fst* for pairwise comparisons between sites (*e.g.*, blue bars indicate average *Fst* among all pairs of recently colonized populations); error bars indicate ± 1 standard deviation. Data points for individual pairwise comparisons are visualized in Figure S2.

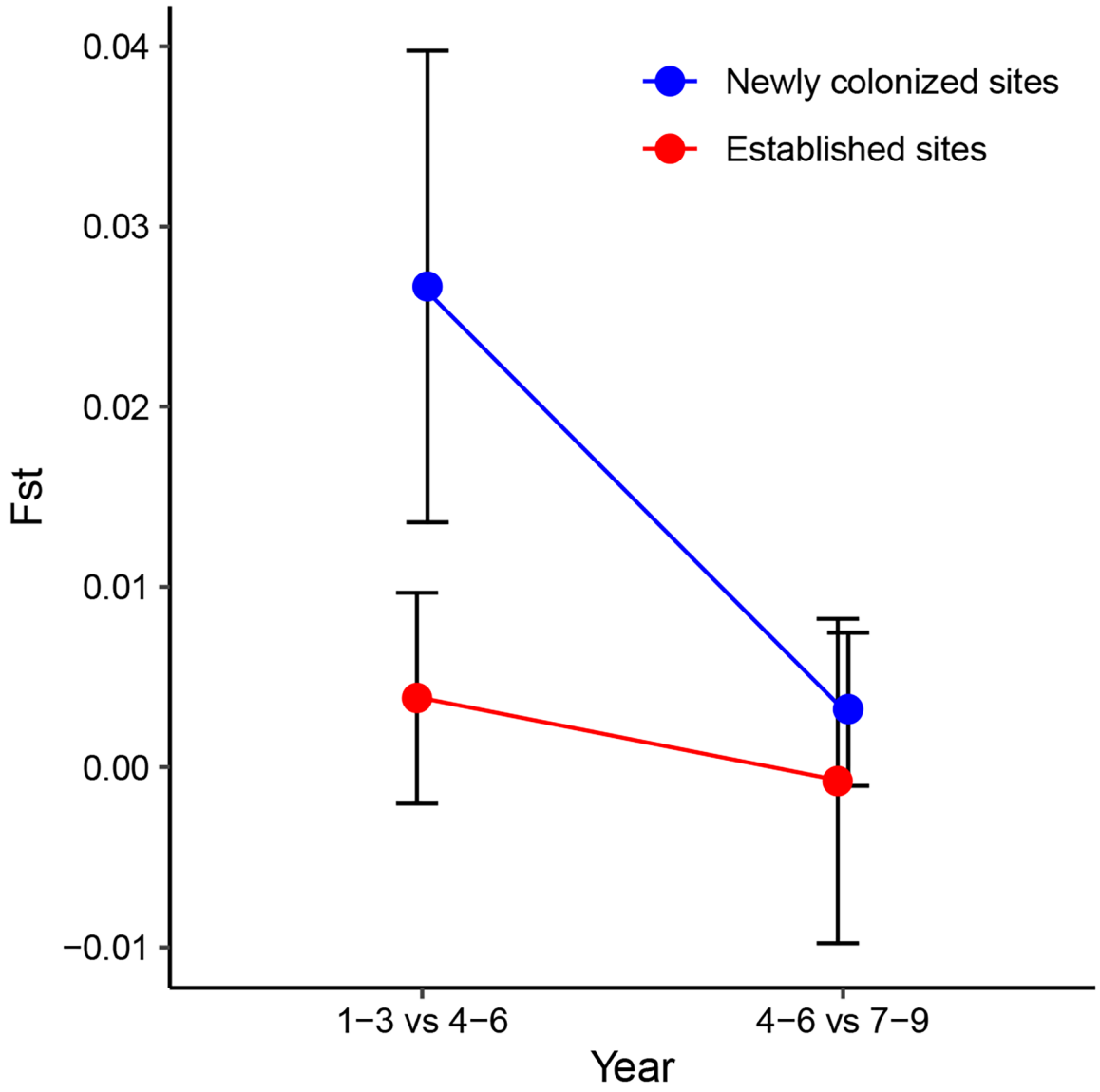

Figure 4. Lineage frequency distributions stabilize 4–6 years post-colonization.

Lineage frequency distributions changed significantly between 1–3 years and 4–6 years post-colonization in recently colonized populations but remained stable among sampling years in long-established populations. However, lineage frequency distributions in recently colonized populations remained stable 4–6 years after colonization. Points indicate average population genetic differentiation (*Fst*), representing changes in lineage frequency distributions across time at each site (*i.e.*, points reflect the degree of within-site change in lineage frequency distributions over time); error bars indicate ± 1 standard deviation.

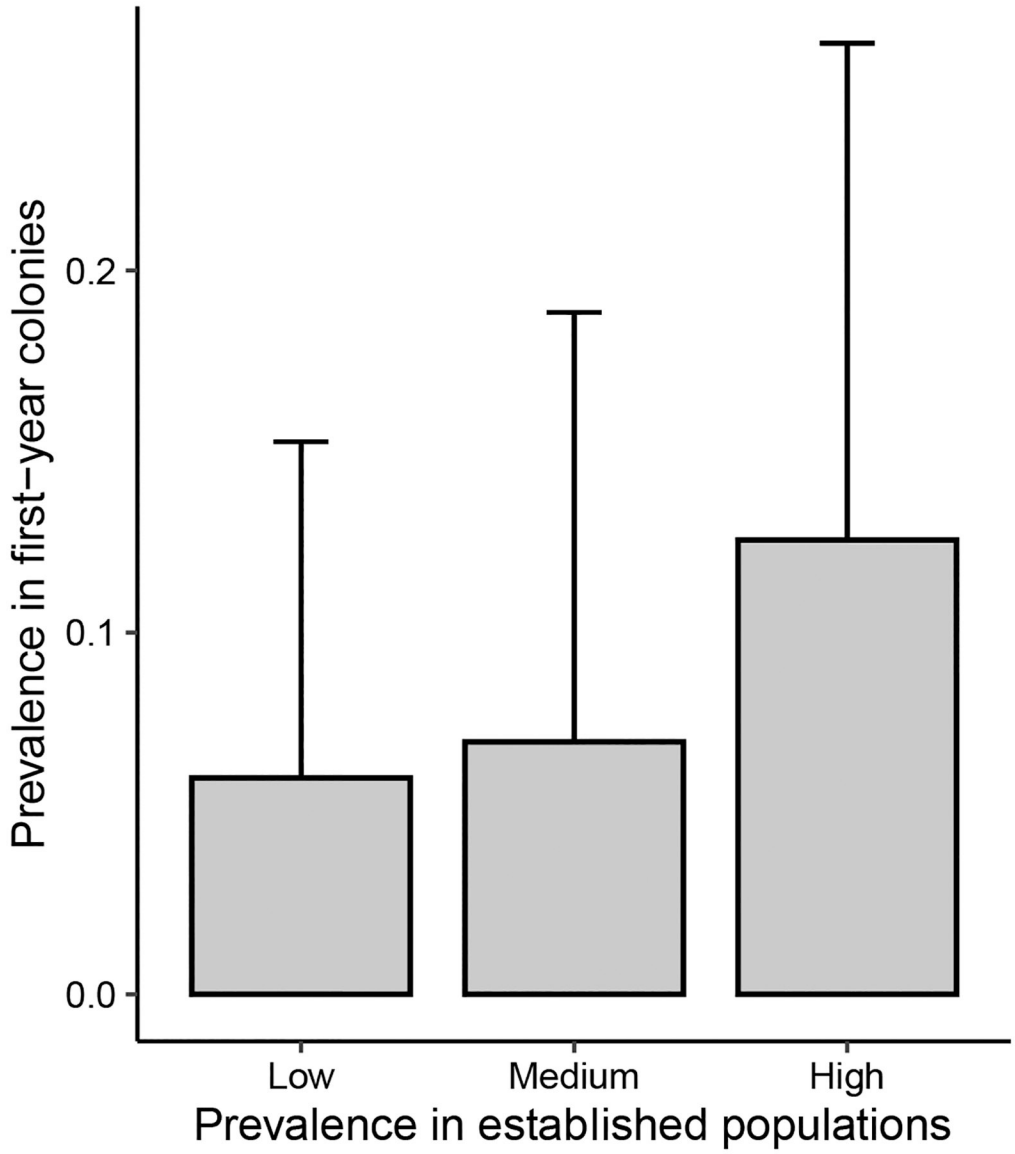

Figure 5. *B. burgdorferi* lineages that are abundant in established populations colonize new populations with higher frequency than rarer lineages.

More prevalent *B. burgdorferi* lineages ("High") were detected more often in newly colonized populations in the first-year post-colonization. Low *B. burgdorferi* lineage prevalence in established populations < 7 %; medium prevalence = 7–14 %; high prevalence > 14 %, where prevalence is the proportion of *Bb*+ ticks infected with that lineage.

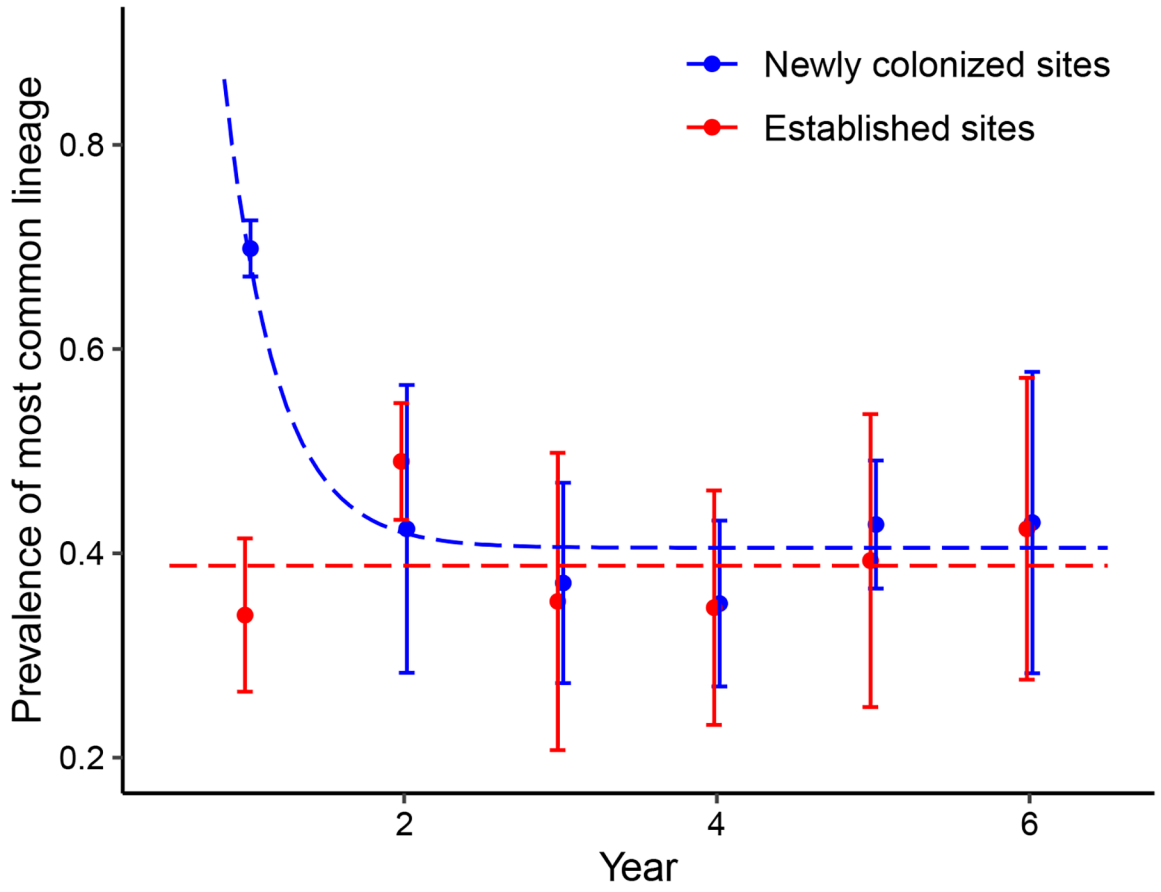

Figure 6. Prevalence of the most common lineage in *B. burgdorferi* populations decreases rapidly post-colonization.

The most common lineage in the year new areas were colonized was significantly more prevalent than the most common lineage in established populations ($p<0.05$). By the second-year post-colonization, the most common lineage in newly established populations was no more prevalent than the most common lineage in established populations. Points are averages; error bars indicate ± 1 standard deviation.