

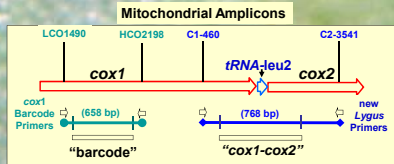
Genetic variation of *Lygus lineolaris* in eastern North America observed with mitochondrial and nuclear DNA

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Lygus lineolaris is the pest *Lygus* species of note in North America from the Great Plains to the Atlantic Coast and from southern Canada to the Gulf of Mexico. DNA sequences from three regions have been used to explore its genetic diversity.

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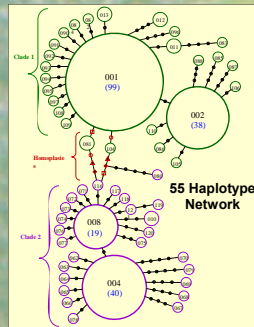


cox1-cox2

MtDNA



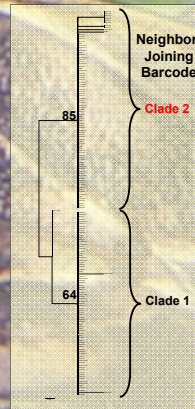
Barcode



Examination of a 768-bp segment of mtDNA spanning the *cox1-cox2* genes recovered 60 haplotypes from 258 insects. Four of the haplotypes are comparatively common while the remaining 56 were observed only once or twice. Phylogenetic trees and haplotype networks reveal two genetic clades with somewhat different geographic distribution. Although the two clades are sympatric, one is much more numerous in the northern part of the species' range.

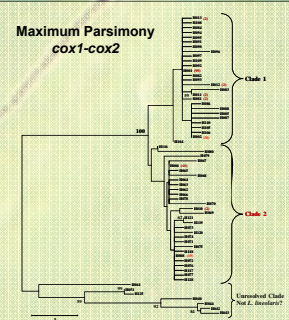
Region	State/province ID (no. of sequences)	Haplotype number(s)
RRV	MN (37)	1, 2, 4, 8, 83, 108, 110
RRV	ND (99)	1, 2, 4, 8, 13, 51, 68, 80, 71, 81, 82, 90, 91, 92, 93, 94, 104, 105, 106
Northeast	CT (7)	4, 8, 42, 43, 41, 80, 85
Northeast	OH (19)	4, 8, 84
Northeast	QC (5)	10, 44, 62, 121
Northeast	VT (10)	1, 2, 4, 8, 80, 98, 118
Gulf Coast	AR (7)	4, 8, 72, 73, 74, 79
Gulf Coast	MS (48)	1, 4, 8, 11, 12, 45, 65, 67, 68, 70, 75, 76, 84, 67, 68, 97, 117, 118, 119, 120, 128
Other	AB (2)	1, 2
Other	SA (2)	1, 4
Other	IL (1)	4
Other	IN (5)	1, 4, 8
Other	MI (2)	2, 109
Other	MO (4)	1, 2, 4, 8, 86, 78, 36
Other	NE (2)	8, 125
Other	SD (5)	1, 2
Other	SK (2)	1, 4

A 658-bp portion of the mitochondrial *cox1* barcode region supports the two clades but lacks the haplotype diversity. It was easier to screen and was employed to provide further details regarding the distribution of the clades.



Neighbor-Joining tree of barcode sequences with the sum of branch length = 0.01381753 is shown. Bootstrap numbers (1000 replicates) are shown next to the branches.

Haplotype Network of *cox1-cox2* 768 bp haplotypes. Circle size proportional to number of individuals in each haplotype (actual number in blue)



Maximum parsimony topology based on *Lygus lineolaris* mtDNA *cox1-cox2* haplotypes (768 bp). Bootstrap values >85% are reported at the appropriate nodes. Clades are indicated on the right of the trees. The tree is consensus of 191 trees [tree length = 143]. Numbers in parentheses are total number of specimens belonging to respective haplotypes. Scale units are number of substitutions. Several haplotypes did not link to the others and are most likely errors, misidentifications or numts.

States and provinces of sample origins and numbers of *L. lineolaris* specimens used in TCS networking of *cox1-cox2* amplicon

Region	Others	Gulf	RRV	NE
Others	0.00450	0.00017	0.00061	0.00068
Gulf	0.00623	0.00762	0.00179	0.00017
RRV	0.00410	0.00683	0.00246	0.00260
NE	0.00938	0.01043	0.01028	0.00129

Estimates of average evolutionary divergence for *cox1-cox2*: within groups (bold diagonal); between groups (below diagonal); net evolutionary divergence between groups (above diagonal).



MtDNA Summary

Both the "barcode" region and a longer *cox1-cox2* separate *L. lineolaris* into two genetic clades

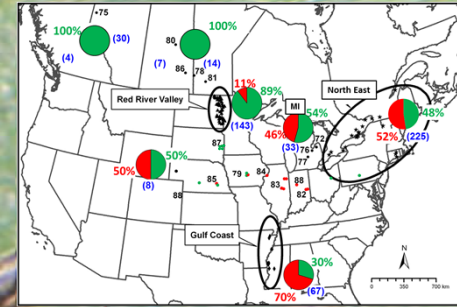
Major haplotypes of clades separated by 3 substitutions (barcode) and 6 substitutions (*cox1-cox2*) - ≈0.5%

Intermediate genotypes are rare

Distribution of clades has geographical component
 ❖ Clade 1 more common north and west
 ❖ Clade 2 more common south and east

Mating barriers unknown

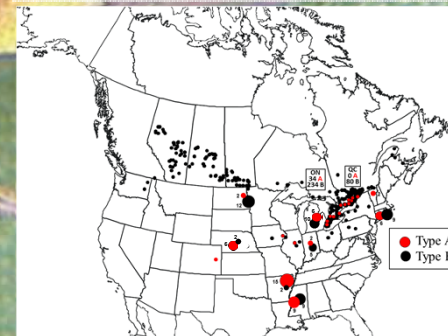
L. lineolaris Regional Distribution of Clade 1 & Clade 2 (combined mitochondrial data)



Barcode and *cox1-cox2* data were merged to show the geographic distribution of Clade 1 and Clade 2. The sample number for provinces, states or regions is in blue. Individuals not included in one of the pie charts are marked by small red or green circles. Clade1 dominates the northern portion of the range while Clade 2 is more common in the south.

LL13 MicroSatellite Flanking Region

A nuclear fragment comprising 452 bases flanking a microsatellite locus (MSFR) separates into two common clades (Type A, n = 73; Type B, n = 420) and a third clade that is rare (Type C, n = 3). The two common clades differ by 10 substitutions. The third clade differs by 12 and 17 substitutions from the other two and is represented by only three individuals. The mitochondrial and nuclear clades are not coincident, although 90% of Clade A MSFR have been mitochondrial Clade 2 and 72% of MSFR Clade B have been mitochondrial Clade 1. There is no apparent link between the mitochondrial or nuclear genotypes and the host plant origins.



MSFR distribution also varies north to south. In Canada Type A was found only in SE Ontario in strip of about 80 km immediately north of the Great Lakes/St. Lawrence River. Type A is rare in the Red River Valley of the North but common in the lower Mississippi River Valley. Type B is widely distributed but appears less frequent in the south. Numbers of each type are indicated in boxes for ON and QC. Larger 2 circles are proportional to sample sizes (number shown)