SUPPLEMENTARY MATERIAL

This file contains supplementary Tables, Figures and Appendices for the article:

Quiroga, R. E., Mathiasen, P., Quiroga, M. P., Fernández, R. J., Díaz, D. G., & Premoli, A. C. (2025). Estructura y diversidad genética en la gramínea disyunta anfitropical *Leptochloa crinita* (ex *Trichloris crinita*) e implicancias para la restauración. Ecología Austral 35:251–268. https://doi.org/10.25260/EA.25.35.2.0.2509

Table S1. Province/state, country and ecological region of the collection sites of *L. crinita* populations in South (SA) and North America (NA).

Tabla S1. Sitios de colecta de las poblaciones en Sudamérica (SA) y Norteamérica (NA), se detallan Provincia/Estado, Pais y región ecológica.

Subcontinent	Population	Province/State, Country	Ecological region*
NA	Bowie	Arizona, USA	Desierto de Chihuahua
NA	Kinney	Texas, USA	Mezquital Tamaulipano
NA	Knox	New Mexico, USA	Desierto de Chihuahua
NA	Lucero	New Mexico, USA	Desierto de Chihuahua
NA	San Simon	Arizona, USA	Desierto de Chihuahua
NA	Tornillo	Texas, USA	Desierto de Chihuahua
NA	White Sands	New Mexico, USA	Desierto de Chihuahua
SA	Amblayo	Salta, Argentina	Monte de Valles y Bolsones
SA	Chamical	La Rioja, Argentina	Chaco Seco
SA	Colpes	Catamarca, Argentina	Monte de Valles y Bolsones
SA	HC	Córdoba, Argentina	Chaco Seco
SA	HL	Córdoba, Argentina	Chaco Seco
SA	RCH Ancha	Mendoza, Argentina	Monte de Llanuras y Mesetas
SA	RCH Fina	Mendoza, Argentina	Monte de Llanuras y Mesetas
SA	Recreo	Catamarca, Argentina	Chaco Seco
SA	Recreo Salinas	Catamarca, Argentina	Chaco Seco
SA	Salinas Grandes	La Rioja, Argentina	Chaco Seco
SA	San Martín	Catamarca, Argentina	Chaco Seco
SA	SC	La Rioja, Argentina	Chaco Seco
SA	SL	La Rioja, Argentina	Chaco Seco
SA	Tilimuqui	La Rioja, Argentina	Monte de Valles y Bolsones
SA	El Tipán	Catamarca, Argentina	Chaco Seco

*according to: Burkart, R., N. Bárbaro, R. O. Sánchez, and D. A. Gómez. 1999. Ecorregiones de la Argentina, APN, PRODIA. Pp. 43.

Olson, D. M., E. Dinerstein, E. D. Wikramanayake, N. D. Burgess, G. V. Powell, et al. 2001. Terrestrial Ecoregions of the World: A New Map of Life on Earth. A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. BioScience 51:933-938.

Table S2. Number of individuals analized per population and subcontinent (NA, North America; SA, South America) for each molecular marker.

Tabla S2. Cantidad de individuos analiszados por población y subcontinente (NA, Norteamérica; SA, Sudamérica) en cada marcados molecular.

Subcontinent	Population	*ITS1-5.8S-ITS2*	*rpl32-trnL*	*Mss8*	*Mss11*	*Mss93*
NA	Bowie	0	1	5	5	5
NA	Kinney	1	1	0	5	4
NA	Knox	1	1	0	4	4
NA	Lucero	1	1	4	3	5
NA	San Simon	1	1	5	5	5
NA	Tornillo	1	1	5	4	5
NA	White Sands	0	1	5	5	5
SA	Amblayo	0	1	2	2	2
SA	Chamical	1	1	5	5	5
SA	Colpes	1	1	4	5	5
SA	El Tipán	1	1	5	5	4
SA	HC	1	0	5	1	5
SA	HL	1	1	0	0	0
SA	RCH Ancha	0	1	0	0	0
SA	RCH Fina	1	1	5	1	5
SA	Recreo	1	1	0	4	4
SA	Recreo Salinas	1	1	5	4	5
SA	Salinas Grandes	1	1	5	5	5
SA	San Martín	0	1	5	1	5
SA	SC	1	1	0	0	0
SA	SL	1	0	5	5	5
SA	Tilimuqui	1	1	5	3	5

Table S3. Pairwise population Fst (above diagonal) and Rst (below diagonal) values from microsatellite data. Subcontinent of origin, South America (SA), North America (NA).

Tabla S3. Valores de Fst (arriba de la diagonal) y Rst (debajo de la diagonal) entre pares de poblaciones, obtenidos con los datos de microsatélites. Subcontinente de origen, Sudamérica (SA), Norteamérica (NA).

Subcontinent	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	SA	NA	NA	NA	NA	NA	NA
Populations	Amblayo	Chamical	RCH Fina	HC	Colpes	Recreo	Recreo Salinas	Salinas Grandes	San Martín	SL	Tilimuqui	El Tipan	Bowie	Kinney	Knox	Lucero	San Simon	Tornillo	White Sands
Amblayo	0.000	0.000	0.453	0.483	0.000	0.611	0.000	0.604	0.467	0.089	0.367	0.420	0.000	0.688	0.779	0.000	0.000	0.000	0.000
Chamical	0.071	0.000	0.509	0.544	0.000	0.677	0.000	0.630	0.533	0.000	0.452	0.521	0.000	0.739	0.791	0.086	0.000	0.000	0.000
RCH Fina	0.648	0.758	0.000	0.000	0.444	0.695	0.288	0.761	0.000	0.546	0.494	0.576	0.440	0.774	0.840	0.137	0.509	0.319	0.440
HC	0.669	0.773	0.000	0.000	0.481	0.700	0.338	0.772	0.000	0.589	0.512	0.583	0.472	0.778	0.844	0.179	0.544	0.365	0.472
Colpes	0.000	0.108	0.656	0.669	0.000	0.561	0.000	0.519	0.472	0.000	0.358	0.444	0.000	0.648	0.667	0.031	0.000	0.000	0.000
Recreo	0.545	0.664	0.643	0.649	0.494	0.000	0.608	0.677	0.693	0.697	0.539	0.368	0.596	0.113	0.034	0.491	0.677	0.635	0.596
Recreo Salinas	0.000	0.093	0.461	0.476	0.108	0.560	0.000	0.575	0.326	0.000	0.374	0.438	0.000	0.680	0.714	0.000	0.000	0.000	0.000
Salinas Grandes	0.122	0.000	0.758	0.773	0.136	0.671	0.094	0.000	0.767	0.659	0.086	0.617	0.436	0.739	0.791	0.516	0.630	0.604	0.436
San Martín	0.668	0.773	0.000	0.000	0.669	0.649	0.476	0.773	0.000	0.578	0.505	0.576	0.463	0.774	0.840	0.167	0.533	0.352	0.463
SL	0.313	0.000	0.765	0.780	0.108	0.666	0.117	0.053	0.780	0.000	0.481	0.549	0.016	0.756	0.815	0.136	0.000	0.019	0.016
Tilimuqui	0.146	0.331	0.190	0.203	0.282	0.543	0.000	0.331	0.203	0.350	0.000	0.462	0.275	0.637	0.646	0.274	0.452	0.392	0.275
El Tipan	0.000	0.105	0.639	0.652	0.000	0.484	0.078	0.132	0.652	0.110	0.252	0.000	0.438	0.399	0.432	0.385	0.521	0.472	0.438
Bowie	0.069	0.000	0.758	0.773	0.114	0.666	0.093	0.000	0.773	0.000	0.331	0.110	0.000	0.671	0.694	0.057	0.000	0.000	0.000
Kinney	0.672	0.763	0.758	0.765	0.570	0.000	0.654	0.768	0.765	0.764	0.647	0.565	0.764	0.000	0.256	0.606	0.739	0.709	0.671
Knox	0.998	0.998	0.862	0.871	0.754	0.082	0.793	0.998	0.871	0.998	0.752	0.752	0.998	0.085	0.000	0.608	0.791	0.748	0.694
Lucero	0.094	0.270	0.172	0.183	0.169	0.380	0.025	0.276	0.183	0.284	0.000	0.141	0.271	0.492	0.565	0.000	0.086	0.000	0.057
San Simon	0.071	0.000	0.758	0.773	0.108	0.664	0.093	0.000	0.773	0.000	0.331	0.105	0.000	0.763	0.998	0.270	0.000	0.000	0.000
Tornillo	0.000	0.109	0.457	0.473	0.117	0.563	0.000	0.110	0.473	0.133	0.000	0.088	0.109	0.658	0.797	0.021	0.109	0.000	0.000
White Sands	0.069	0.000	0.758	0.773	0.114	0.666	0.093	0.000	0.773	0.000	0.331	0.110	0.000	0.764	0.998	0.271	0.000	0.109	0.000

Table S4. Medians of the estimated parameters for the selected scenarios of separation between *L. crinita* populations of South and North America. RMAE (relative median absolute error) value for each parameter is shown in parentheses, as an indicator of its precision.

Tabla S4. Medianas de los parámetros estimados para los escenarios seleccionados de separación entre las poblaciones de *L. crinita* de Sudamérica y Norteamérica. Para cada parámetro se muestra el valor de RMAE (mediana relativa del error absoluto) como indicador de su precisión.

	Most likely scenario [logistic approximation probability]	SA*	NA	t1	useq	k1seq	PA	Âμmic	pmic	snimic
rpl32-trnL	Scenario 4 [0.25, 0.23-0.28]	37500 (0.24)	52700 (0.26)	3330 (0.22)	4.1×10^{-8} (0.20)	7.9 (0.05)	-	-	-	-
ITS1-5.8S-ITS2	Scenario 1 [0.67, 0.57-0.77]	7930 (0.24)	4000 (0.30)	2040 (0.21)	6.9×10^{-8} (0.32)	5.4 (0.14)	-	-	-	-
Microsatellites	Scenario 6 [0.38, 0.36-0.39]	48700 (0.24)	36800 (0.25)	462 (0.27)	-	-	986 (0.12)	1.8×10^{-4} (0.22)	2.4×10^{-1} (0.06)	6.4×10^{-8} (0.61)

*Abbreviations: SA and NA, are the effective population sizes for South America and North America, respectively;
t1, is the time –in number of generations- since the divergence or separation of the populations of South and North America, for *L. crinita* a time between generations of 6 years was estimated (see Dsicussion);
PA, is the effective population sizes of the ancestral population;
useq, is the sequence mutation rate;
k1seq, is the haplotipe number;
μmic, is the microsatellite mutation rate;
pmic, is the proportion of mutations larger than one step;
snimc, is the non-standard mutation rate.

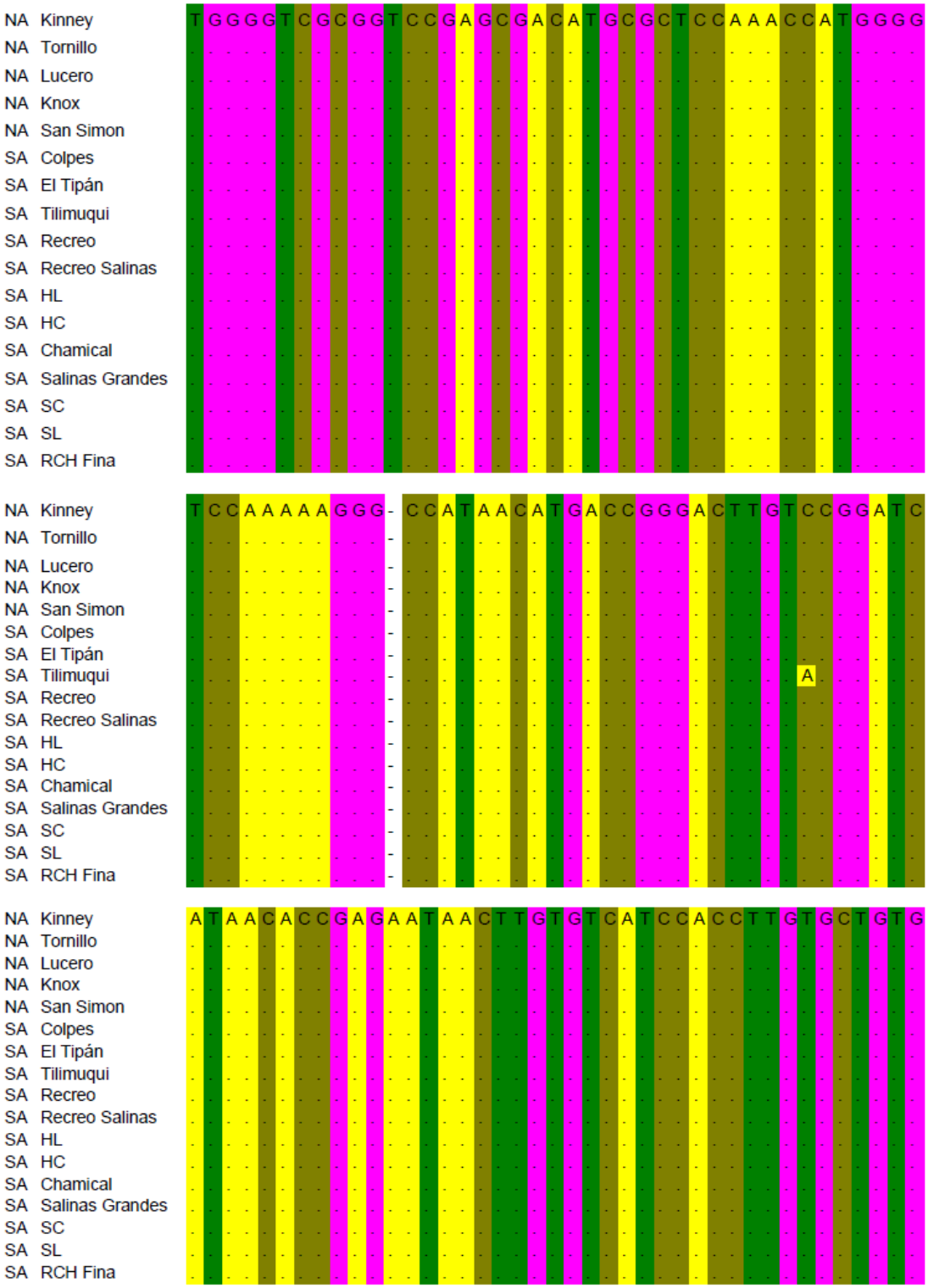
Table S5. Present and past (Middle Holocene, 6000 years ago; Last Glacial Maximum, 21000 years ago) average values (± standard deviation) of mean annual temperature (AMT) and annual precipitation (AP) of sites actualy inhabited by *L. crinita* in South and North America (coordinates obtained from www.gbif.org). Climatic data was obtained from the Worldclim 1.4 database (www.worldclim.com).

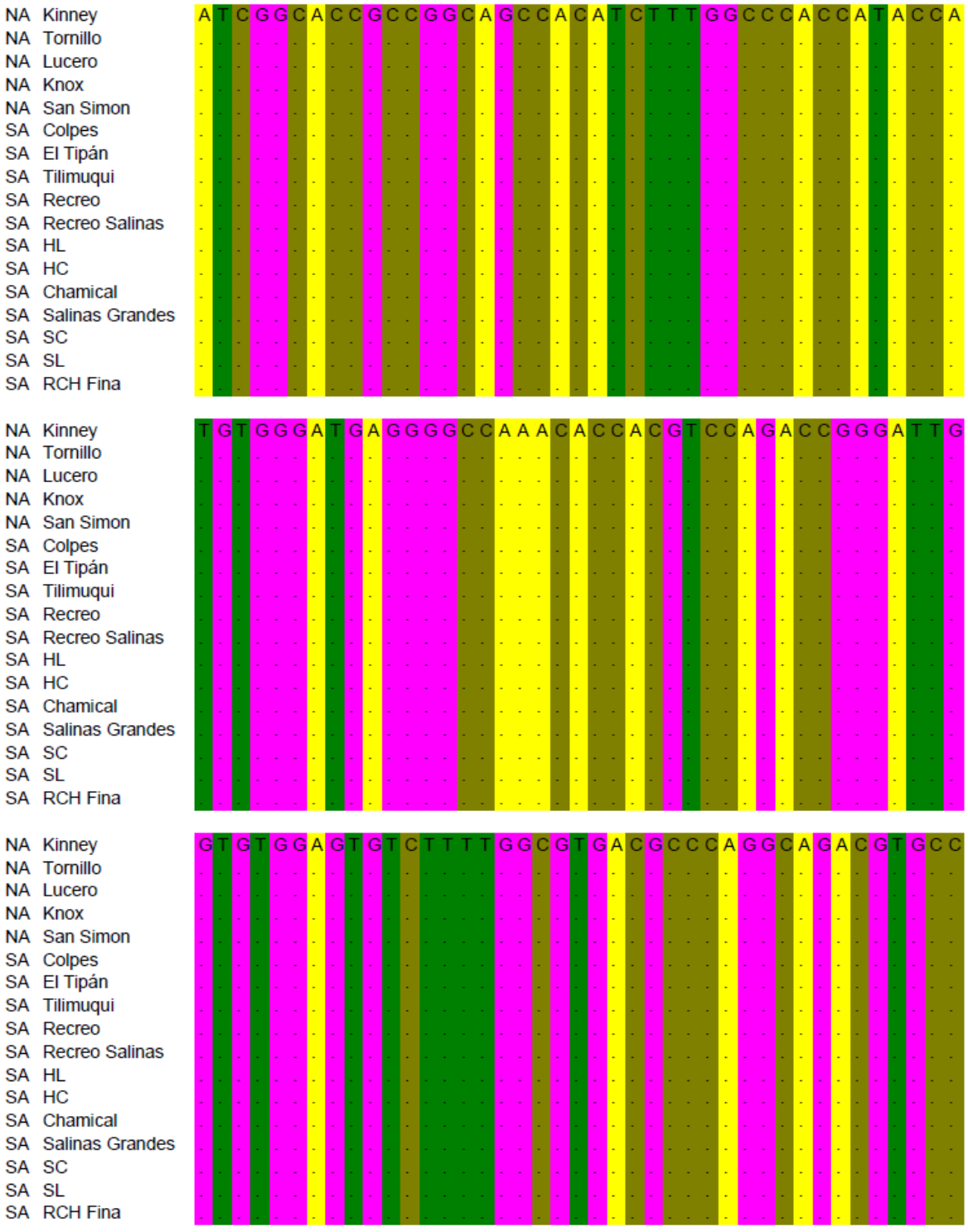
Tabla S5. Valores promedio correspondientes al presente y al pasado (Holoceno Medio, hace 6000 años; Último Máximo Glacial, hace 21000 años) de temperatura media anual (TMA) y precipitación anual (AP) en sitios que habita actualmente *L. crinita* en Sudamérica y Norteamérica (coordenadas obtenidas de www.gbif.org). Los datos climáticos fueron obtenidos de la base Worldclim 1.4 (www.woldclim.com).

Subcontinent	Present		Middle Holocene		Last Glacial Maximum	
	MAT (°C)	AP (mm)	MAT (°C)	AP (mm)	MAT (°C)	AP (mm)
South America	18.4	558	17.9	510	15.1	481
	(3.3)	(307)	(3.3)	(286)	(3.3)	(322)
North America	19.9	321	18.8	347	13.9	341
	(2.6)	(136)	(2.6)	(139)	(3.4)	(108)

Figure S1. Alignment of the *ITS1-5.8S-ITS2* sequence, for different populations of *L. crinita*. The subcontinent of origin (NA, North America; SA, South America) of each population are showed.

Figura S1. Alineamiento de la secuencia *ITS1-5.8S-ITS2*, para distintas poblaciones de *L. crinita*. Se detalla el subcontinente de origen (NA, Norteamérica; SA, Sudamérica) de cada población.

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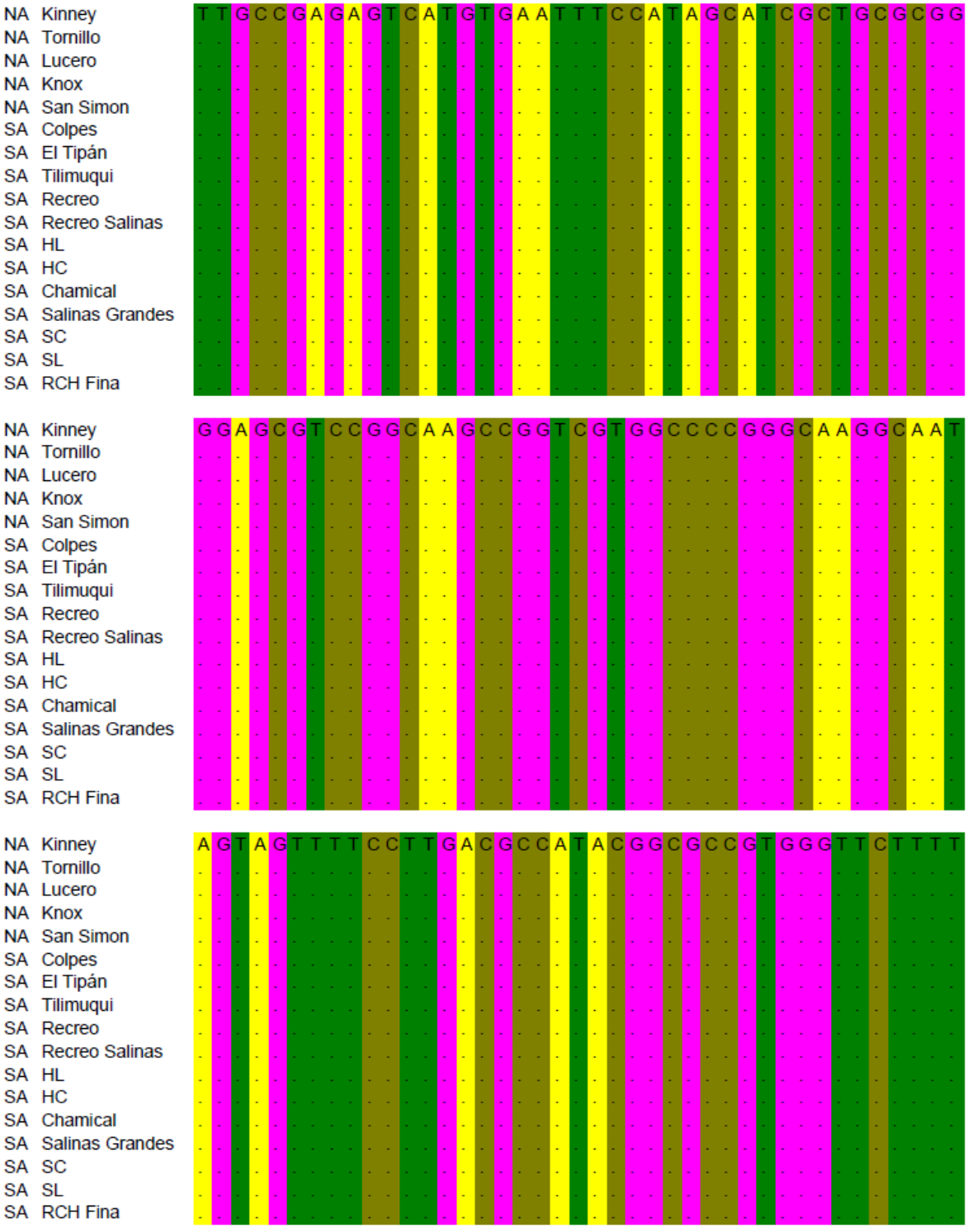
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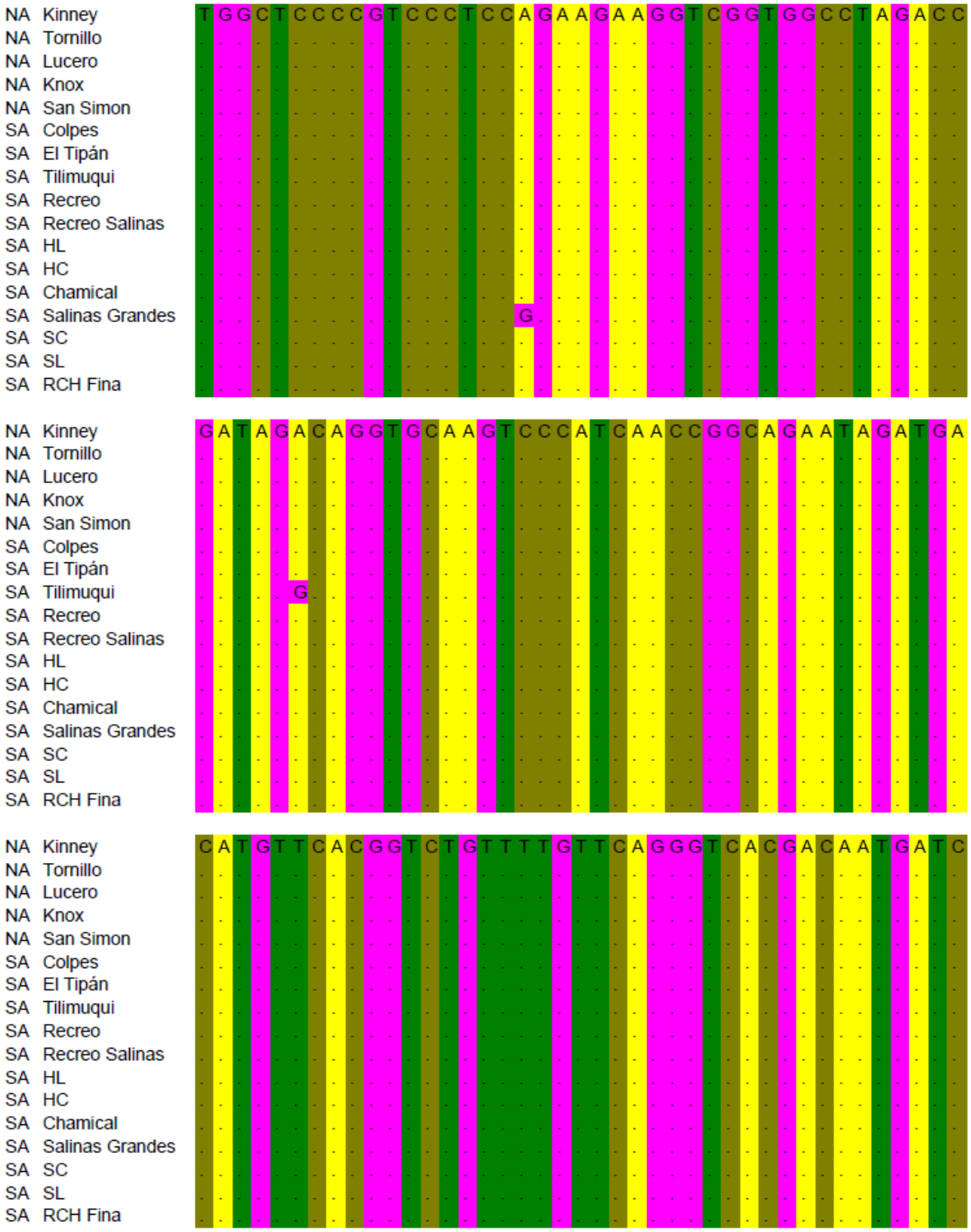
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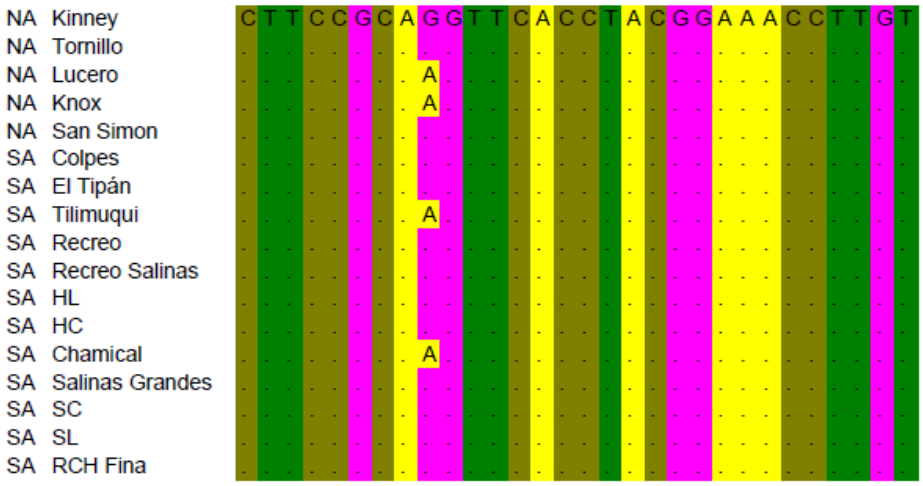
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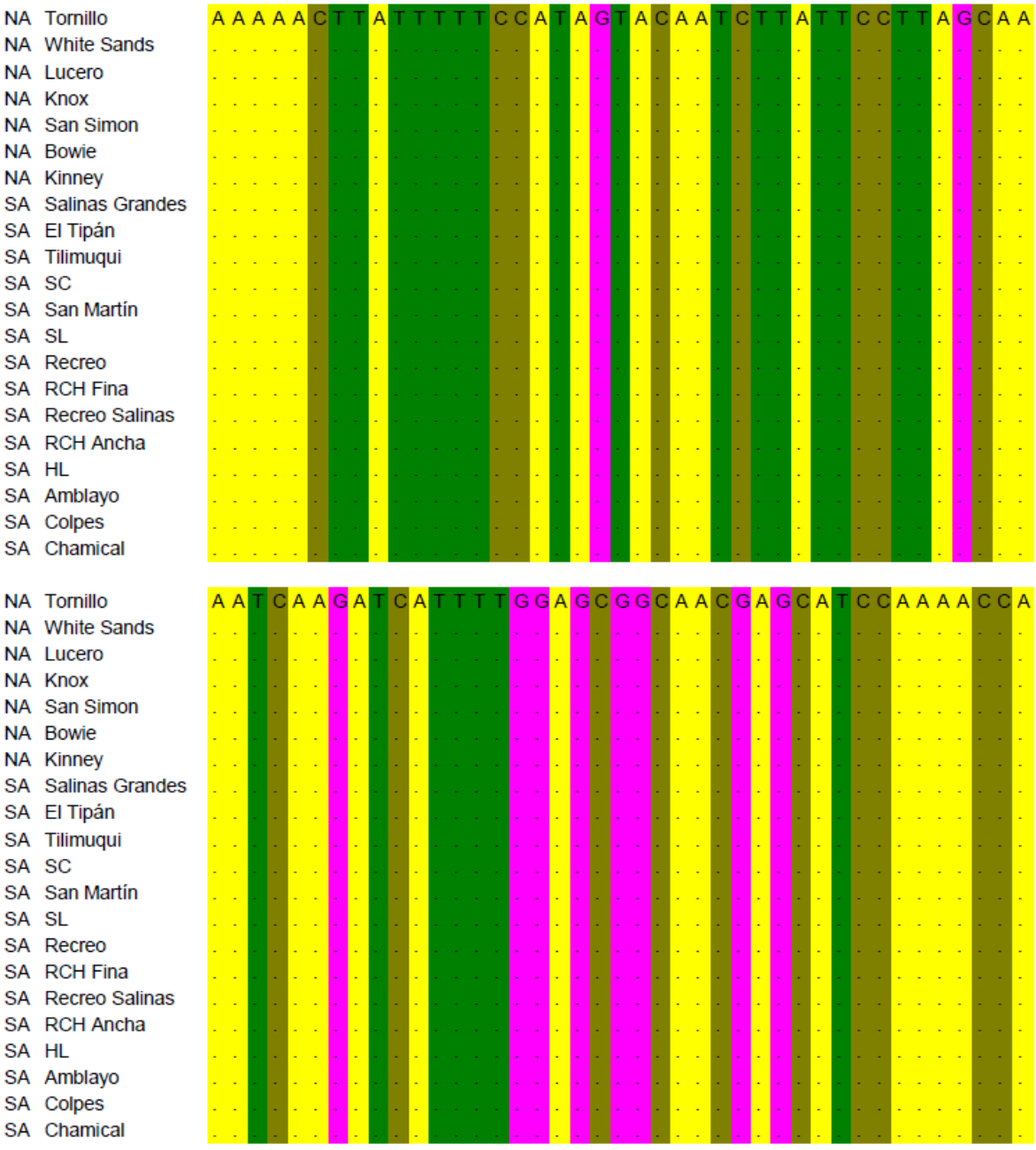
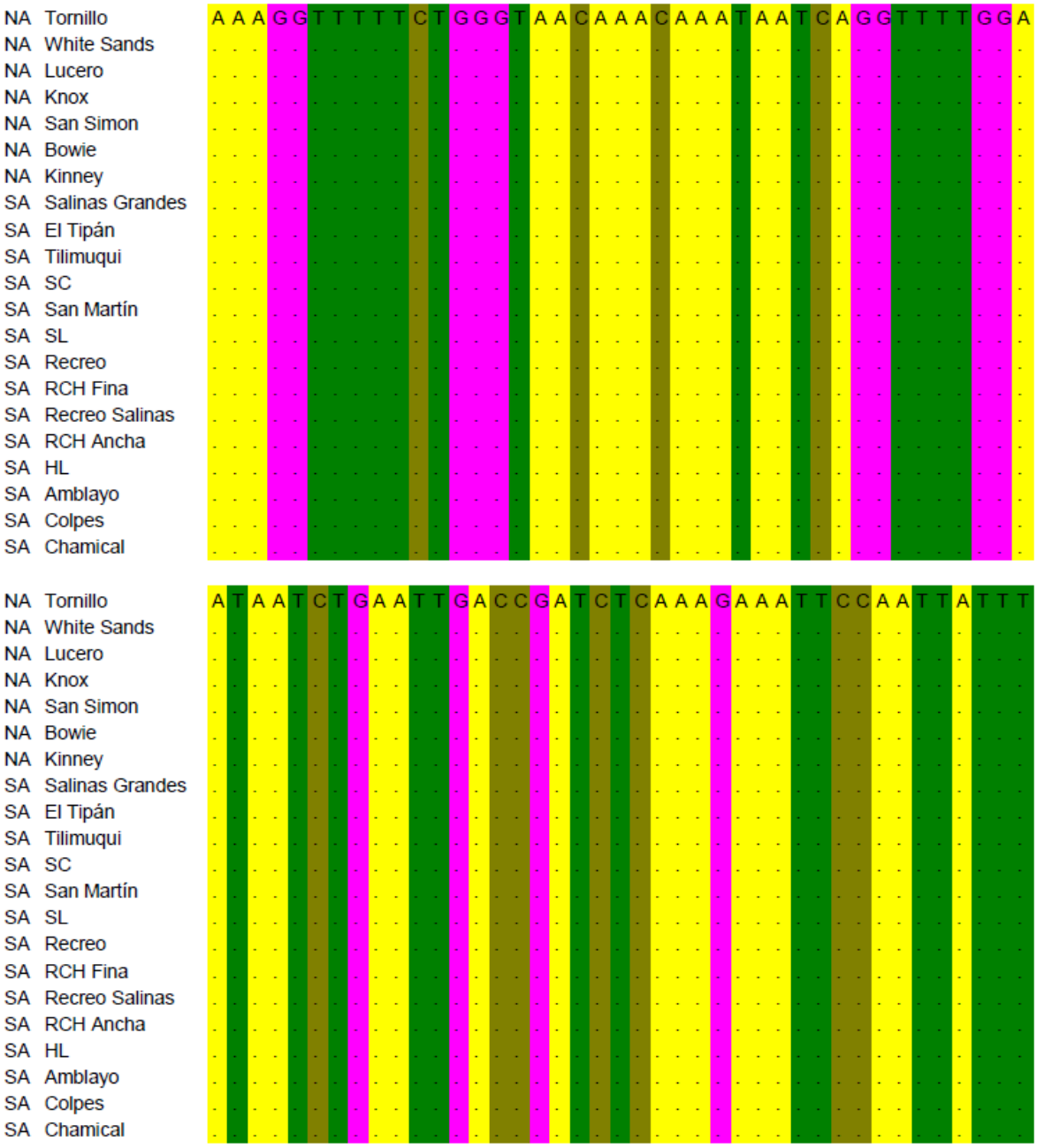
C T T C C G C A G G T T C A C C T A C G G A A A C C T T G T

Figure S2. Alignment of the *rpl32-trnL* sequence, for different populations of *L. crinita*. The subcontinent of origin (NA, North America; SA, South America) of each population are showed.

Figura S2. Alineamiento de la secuencia *rpl32-trnL,* para distintas poblaciones de *L. crinita*. Se detalla el subcontinente de origen (NA, Norteamérica; SA, Sudamérica) de cada población.

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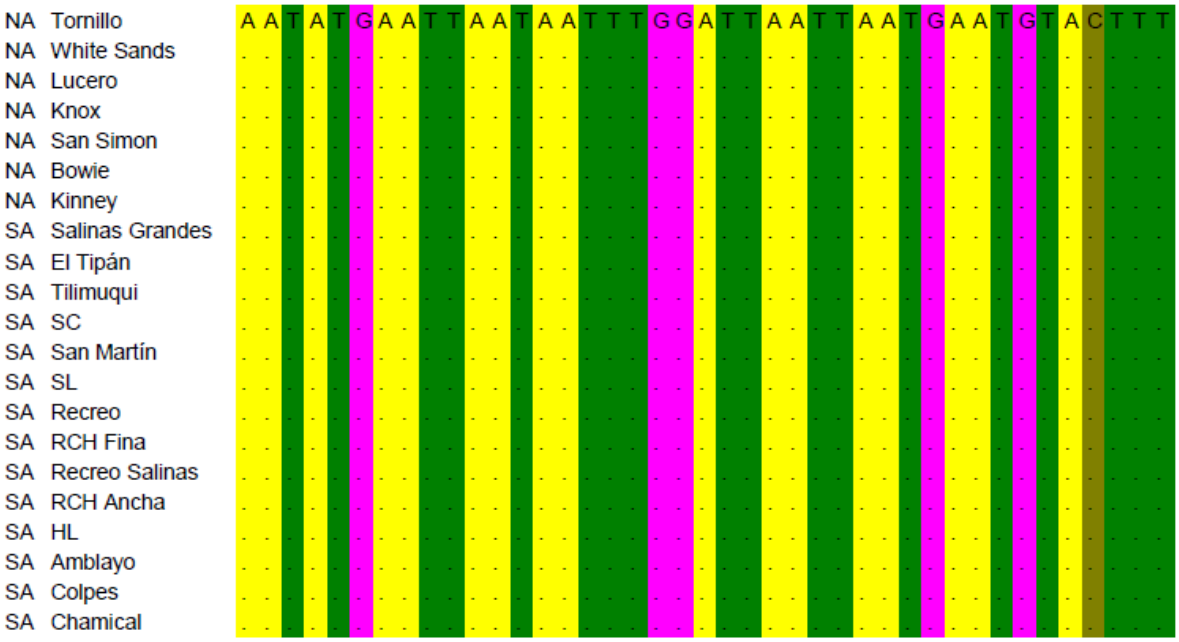
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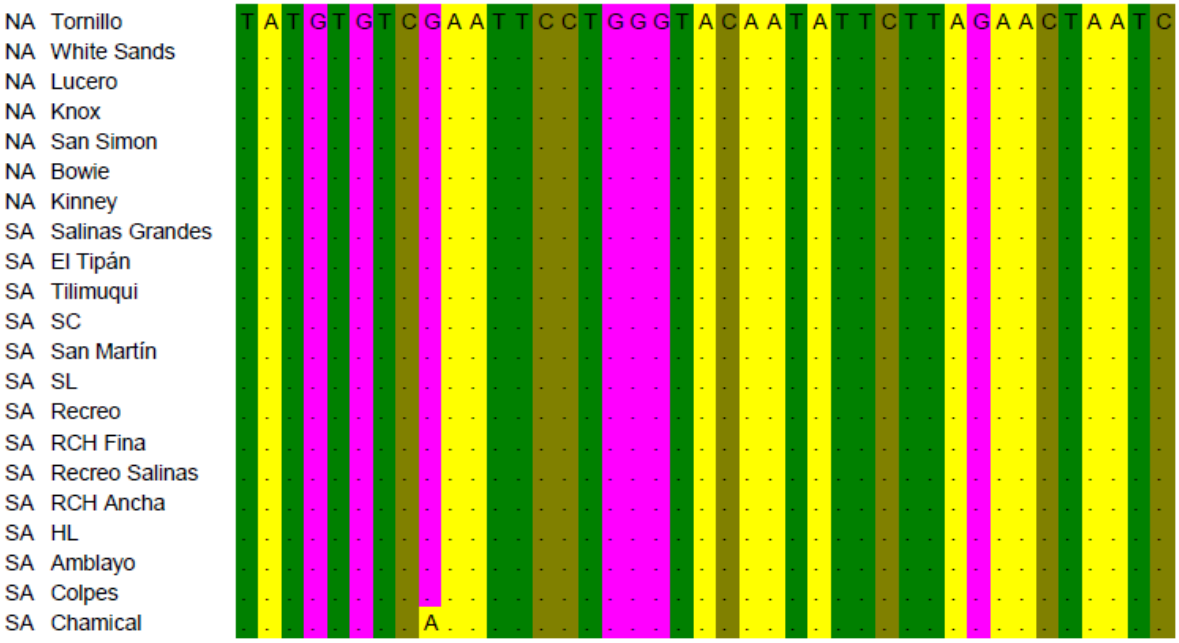
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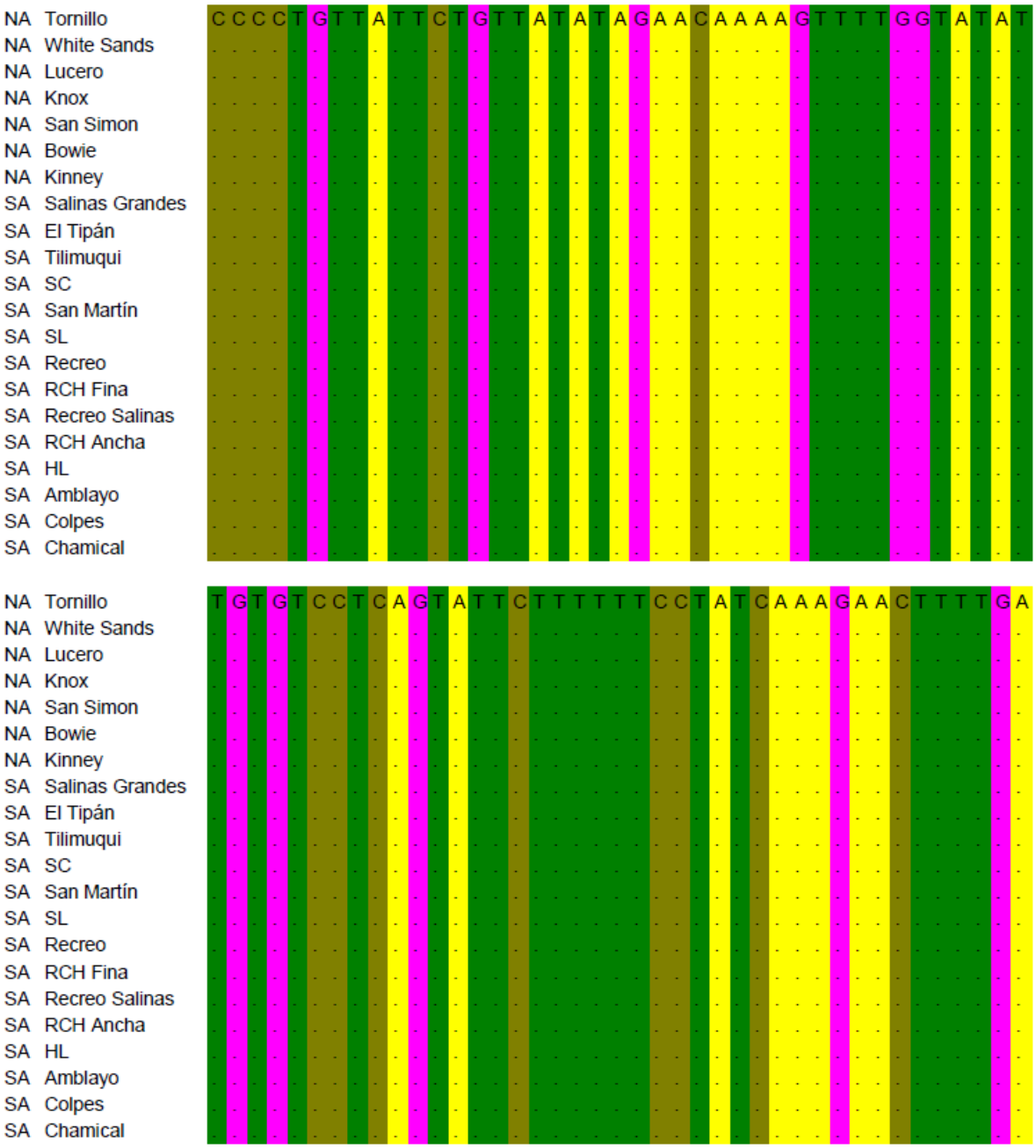
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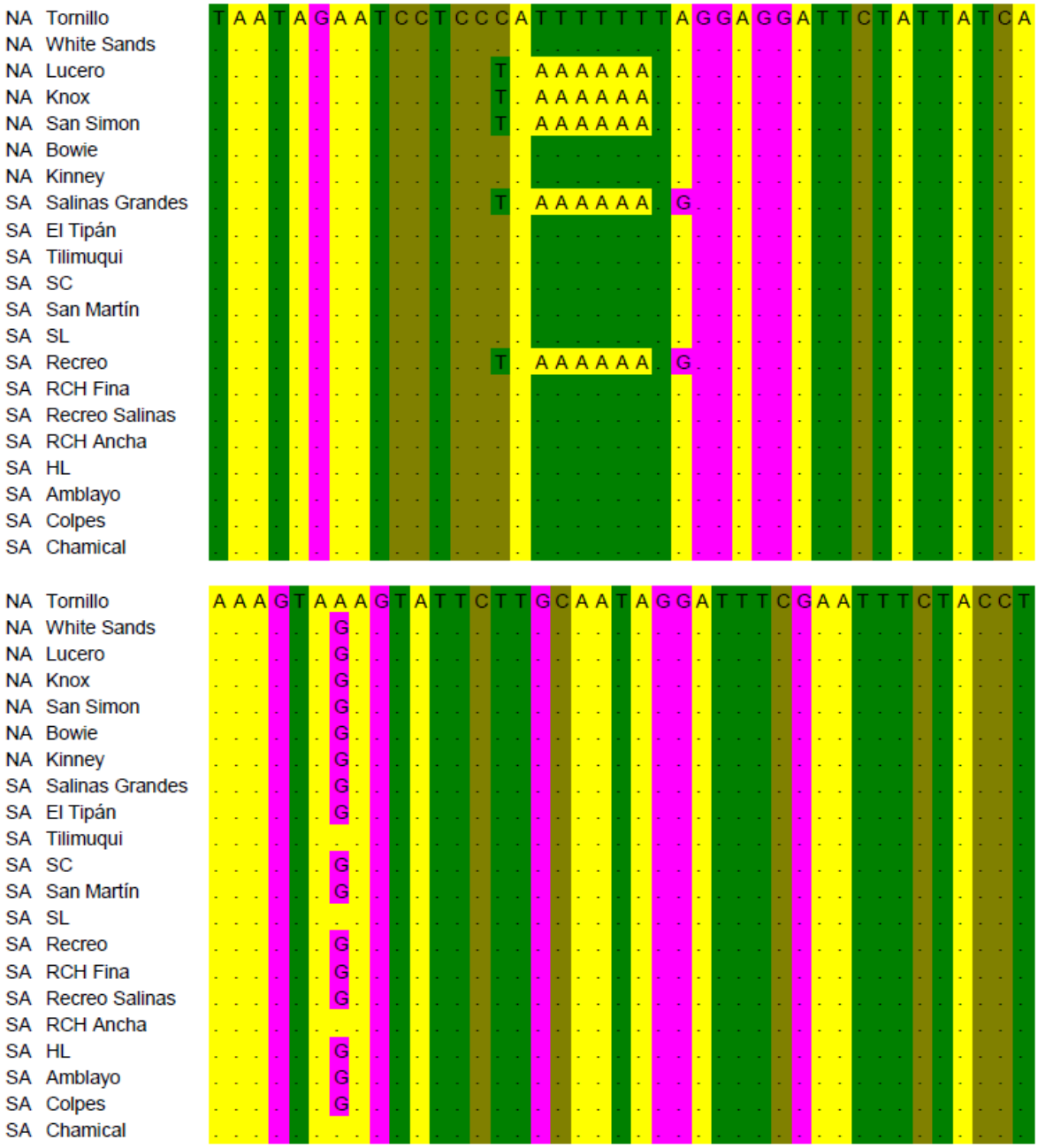
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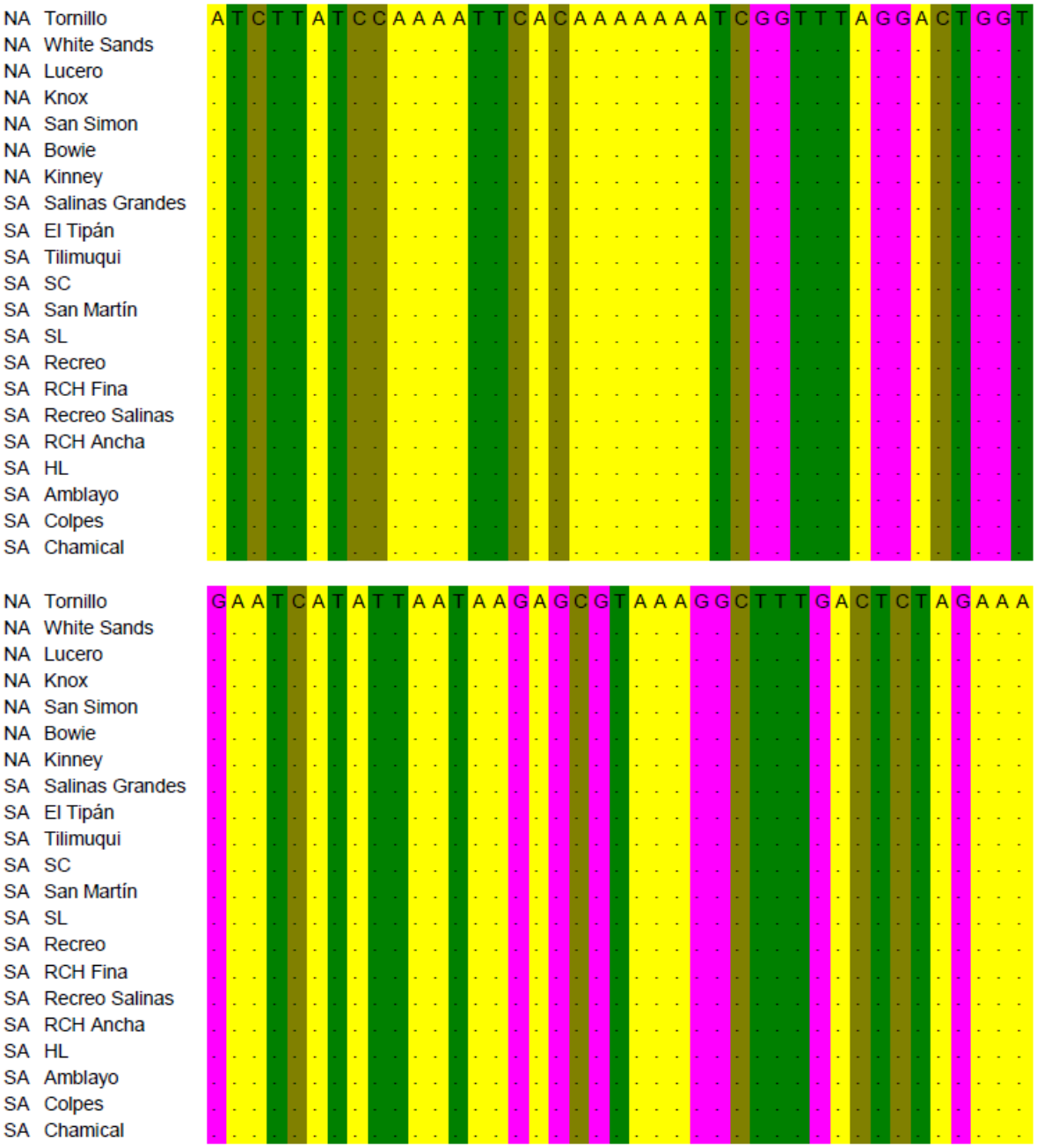
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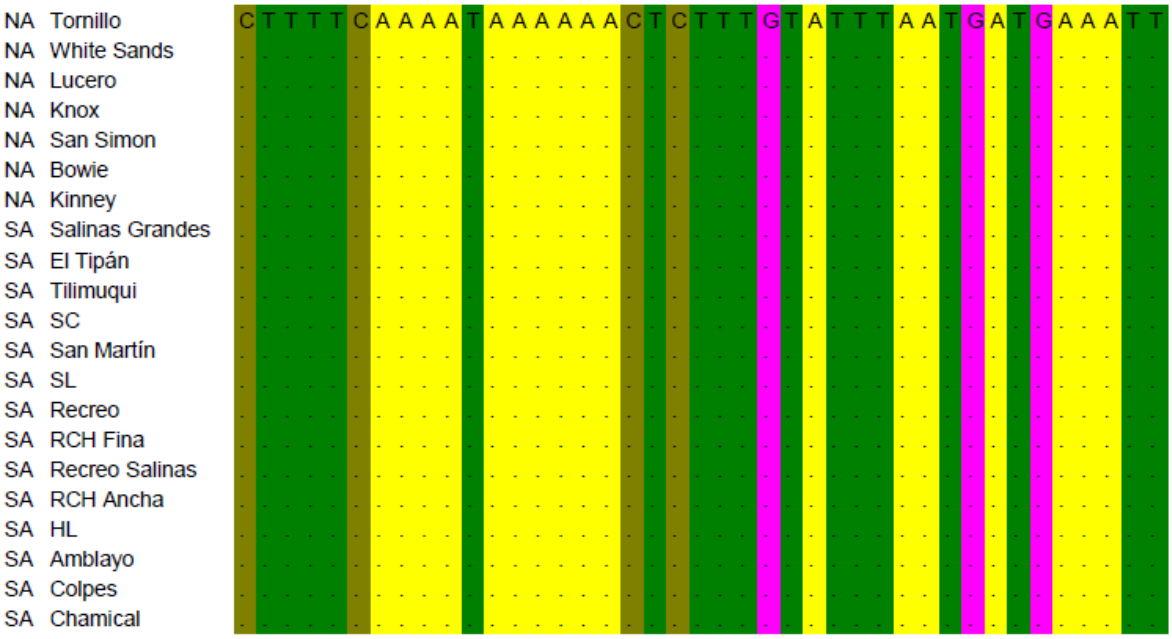
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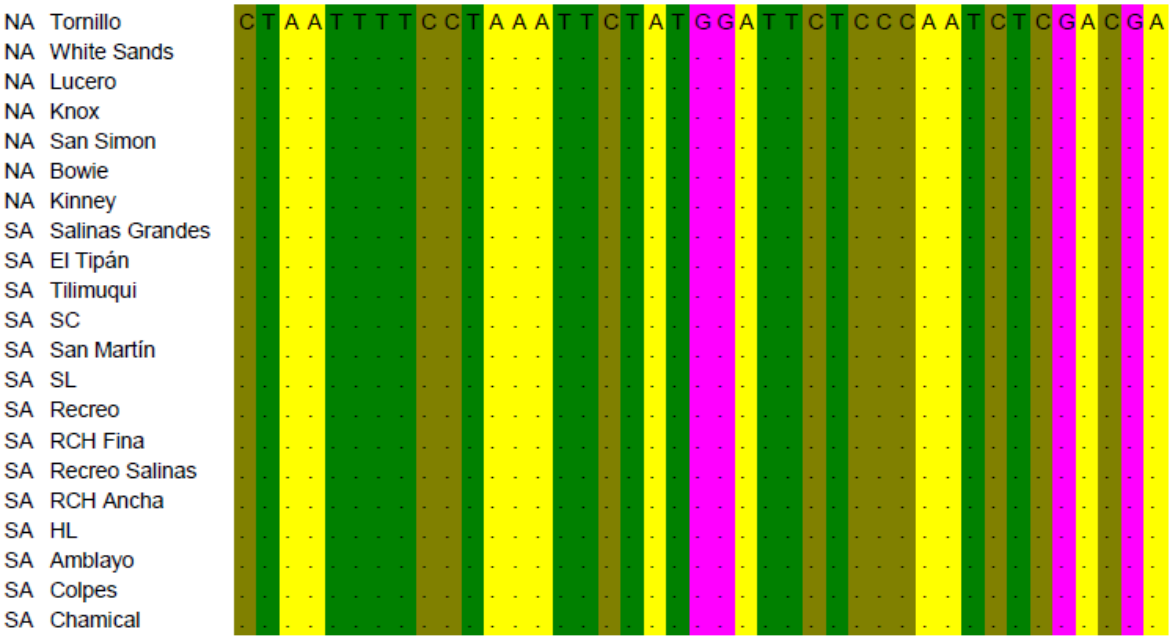
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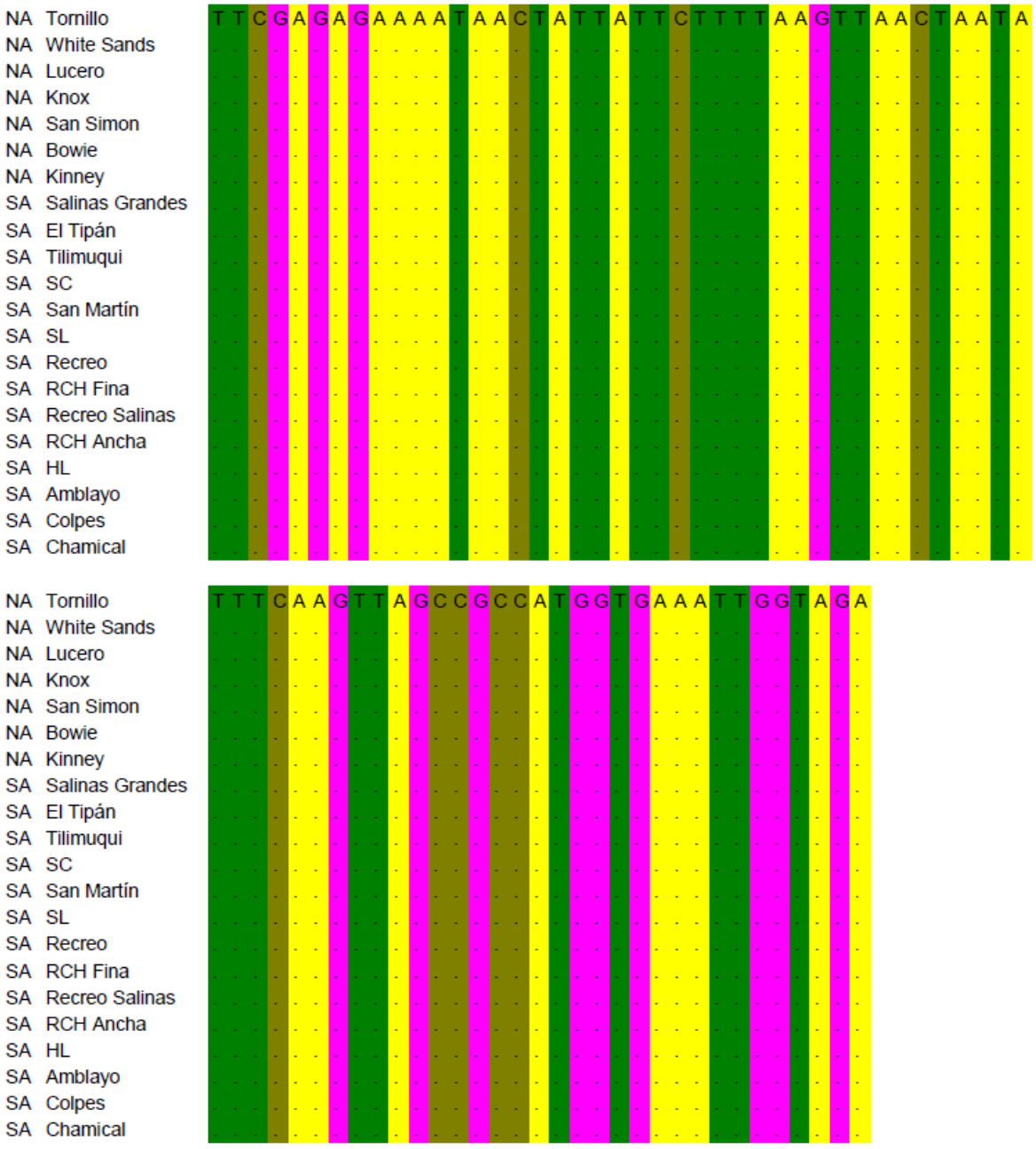
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Figure S3. Relationship between genetic and geographic distances (Mantel test with microsatellite data) among *L. crinita* populatinos of North America (above) and South America (below).

Figura S3. Relación entre las distancias genéticas y geográficas (test de Mantel con datos de microsatélites) entre poblaciones de *L. crinita* de Norteamérica (arriba) y Sudamérica (abajo).

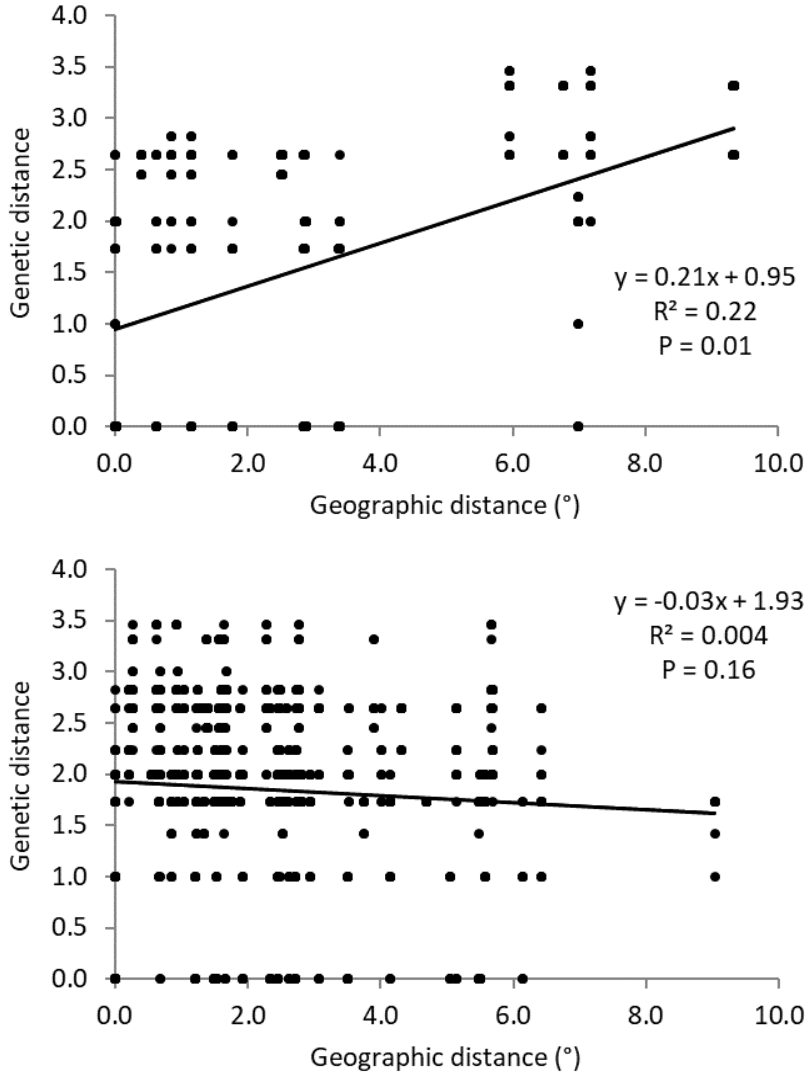


Figure S4. Phylogenetic trees (NJ method) of *L. crinita* populations for microsatellite data (above) and sequences, *ITS1-5.8S-ITS2* (below, left) and *rpl32-trnL* (below, right). North American populations underlined.

Figura S4. Árboles filogenéticos (método NJ) de poblaciones de *L. crinita* según datos de microsatélites (arriba) y secuencias, *ITS1-5.8S-ITS2* (abajo, izquierda) y *rpl32-trnL* (abajo, derecho). Poblaciones norteamericanas subrayadas.

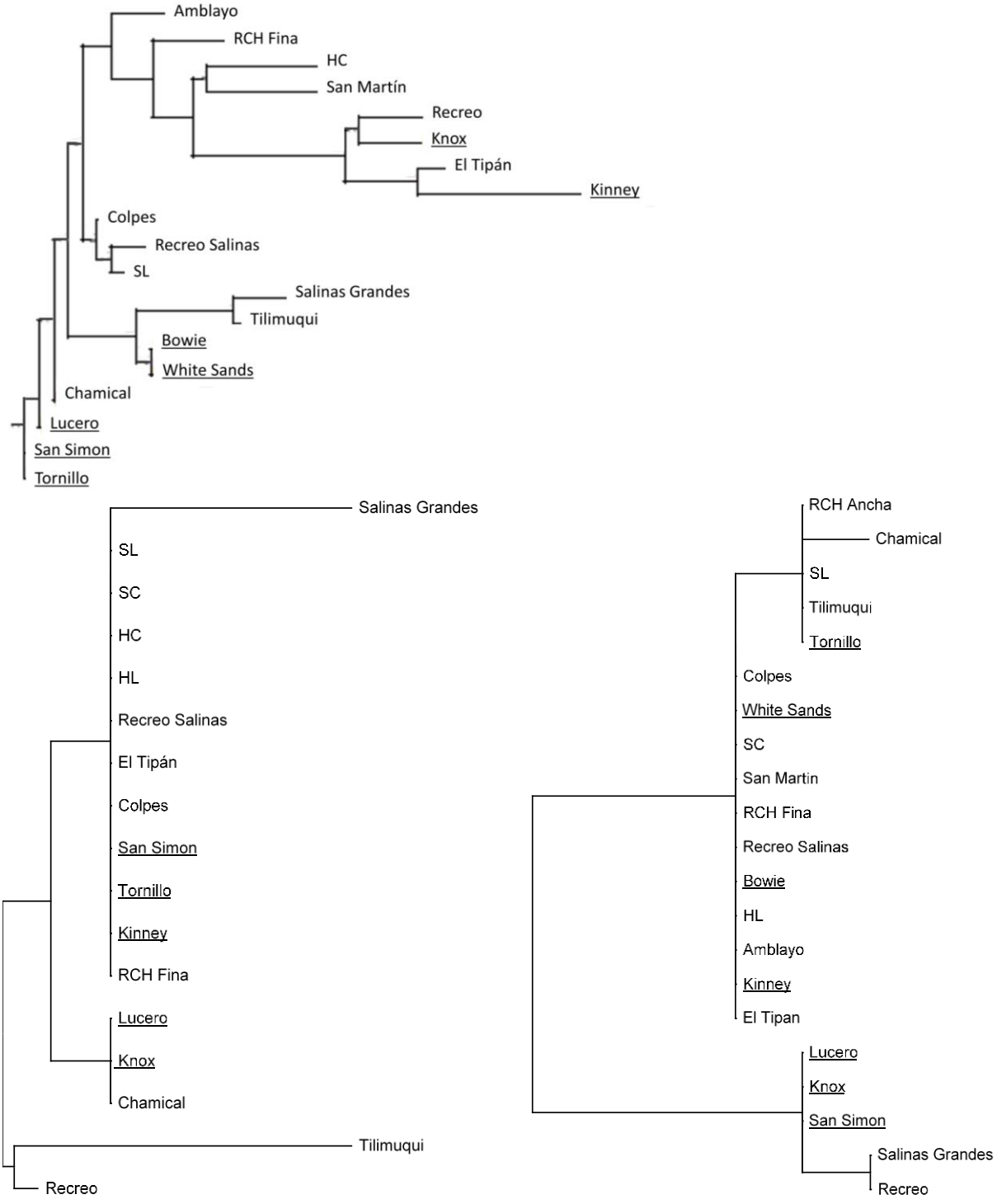
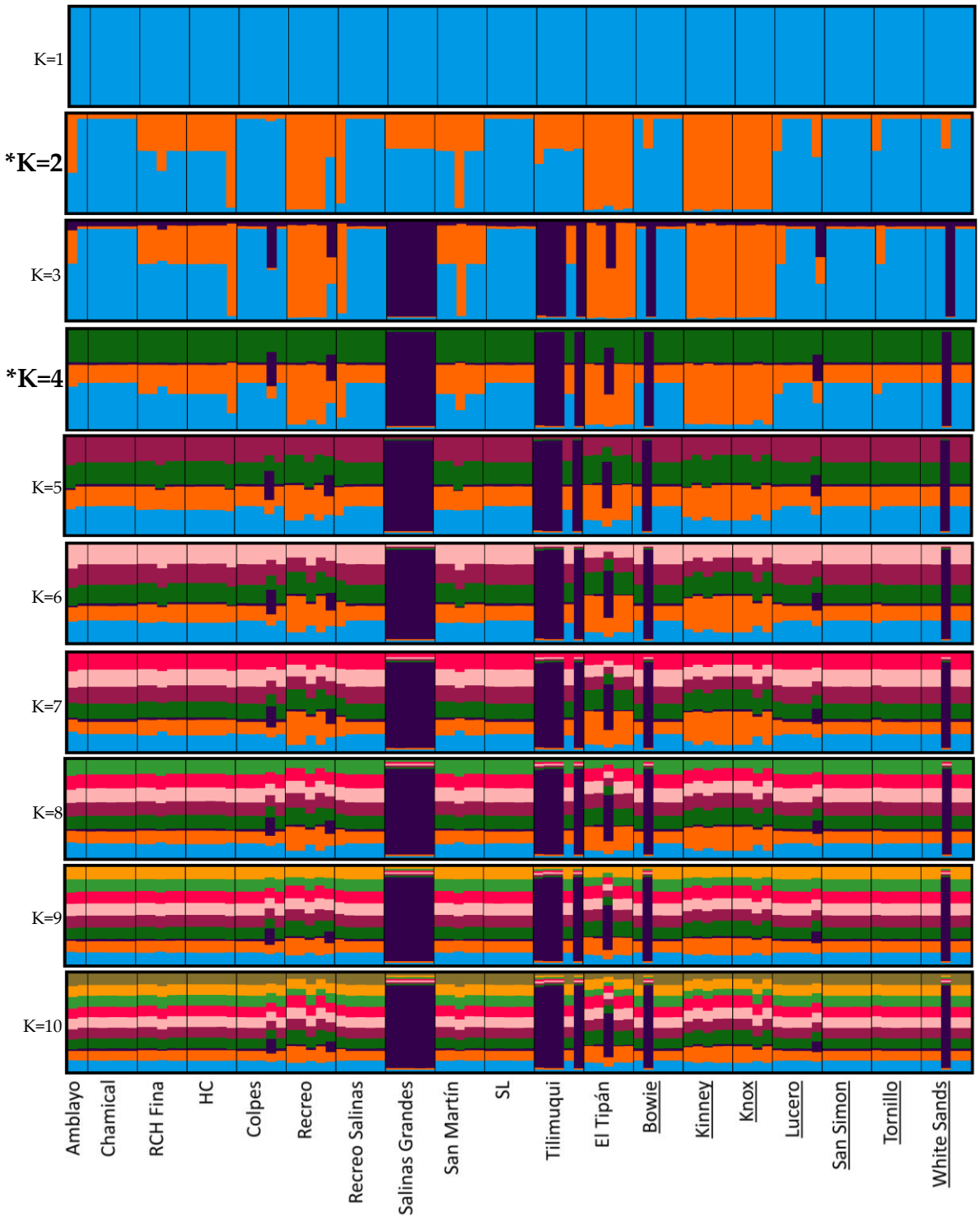


Figure S5. Classification of individuals of different populations of *L. crinita* in genetic groups (K=1to10) with microsatellite data. *Selected groups K=2 and K=4. North American populations underlined.

Figura S5. Clasificación de individuos de las poblaciones de *L. crinita* en grupos genéticos (K=1 to 10) con datos de microsatélites. *Grupos seleccionados K=2 y K=4. Poblaciones norteamericanas subrayadas.



Appendix 1. Laboratory protocols for genetic analyses of *Leptochloa crinita* populations.

Apéndice 1. Protocolos de laboratorio para análisis genéticos de poblaciones de *Leptochloa crinita*.

DNA extraction (adapted from: Doyle, J. J. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.)

RT: room temperature; R: refrigerator; F: freezer.

1. Add 7.5 mg of DTT (R) and 0.01 g of PVP 40000 (RT) for every 1 mL of extraction buffer. Pre-heat the extraction buffer to 55 °C.
2. Add 40 mg of dry leaf material (or 100 mg of fresh leaves) in a 1.5 mL Eppendorf tuve and frozen with liquid nitrogen and grind it to a fine powder.
3. Add 1 mL of extraction buffer to each tube and mix well.
4. Incubate in a hot bath at 55 °C for 1 hour. Homogenize by inverting the tube every 15 min.
5. Remove the tubes from the hot bath and leave at room temperature for 10 min.
6. Add 400 μL of dichloromethane (RT) and mix gently until you get an emulsion.
7. Centrifugate for 10 min at 13000 rpm. Pass the supernatant to a new tube taking care not to pipet the interface.
8. Repeat steps 6 and 7.
9. Add 400 μL (2/3 volume) of isopropanol (F) and mix gently. If the DNA pellet does not appear, put the tubes in the freezer at -20 °C during 30 min (you can leave it overnight).
10. Centrifugate for 15 min at 13000 rpm. Remove the isopropanol taking care of not loosing the pellet and dry the tubes for one hour in filter paper at RT.
11. Add 300 μL of TE or Ae buffer (with RNAse, 0.2 μL/mL) and 200 μL (2/3 vol.) of NaCl 5 M. Mix well and gently until the pellet dissolves.

12. Add 1 mL (2 vol.) of ethanol 100% (R), mix gently. Put the tubes at -20 °C at least 1 hour (you can leave it overnight or several days).

13. Centrifugate for 15 min at 13000 rpm. Carefully remove the supernatant and dry the tubes for one hour in filter paper at RT.

14. Add 50-100 µL of buffer TE 1X (TA) o buffer Ae (TA) con ARNasa (0.2 µL/mL de solución stock: 100 mg/mL). incubate in hot bath at 55 °C for 10 min.

DNA extraction Buffer

ATMAB (Alkyltrimethyl Ammonium Bromide) – Sigma M7635 20 g

*EDTA 0.5 M pH 8 40 mL

*Tris HCl 1 M pH 8 100 mL

*NaCl 5 M 280 mL

Rise to 1000 mL with distilled H_2O

* Autoclave before preparing the buffer. Dissolve the ATMAB in a hot bath at 55 °C. Autoclave the extraction buffer once prepared.

TE Buffer 10X (Stock solution)

Tris 100 mM 12.11 g

EDTA 10 mM 3.72 g

distilled H_2O 1000 mL

Add 20 mg/mL of RNAse

NaCl 5 M Solution (to prepare 1000 mL)

NaCl 292.2 g

distilled H_2O 1000 mL

PCR Conditions for DNA sequences

PCR Mix

H2O	13.72 µL
Buffer 10X	2 µL
DNTPs	0.4 µL (2.5 mM each)
Magnesium	1.6 µL (50 mg)
Primer Foward	0.6 µL (5 µM)
Primer Reverse	0.6 µL (5 µM)
TAQ (Genbiotech)	0.08 µL (5 U/µL)
DNA	0.8 µL (30 ng/µL)
Total volume	20 µL

PCR Conditions

Initial denaturation		94 °C	1 min
30 cycles of:	Denaturation	94 °C	45 s
	Annealing	temperature (varies for each primer)	30 s
	Extension	72 °C	1:30 min
Final extension		72 °C	10 min

PCR Conditions for microsatellites

PCR Mix

H_2O	6.3 µL
Buffer 10X	1 µL
DNTPs	0.8 µL (2.5 mM each)
Primer Foward	0.25 µL (5 µM)
Primer Reverse	0.25 µL (5 µM)
TAQ (Genbiotech)	0.15 µL (5 U/µL)
ADN	1.25 µL (30 ng/µL)
Total volume	10 µL

PCR Conditions

Initial denaturation		94 °C	30 s
40 cycles of:	Denaturation	94 °C	45 s
	Annealing temperature		30 s
	Extension	72 °C	1.5 min
Final extension final		72 °C	10 min

Microsatellite	Annealing temperature	Expected size (bp)	Fluorochrome
Mss8	50 °C	130	NED
Mss11	54 °C	150	VIC
Mss93	54 °C	180	PET
Mss72	57 °C	400	VIC
Mss77	59 °C	650	NED
Mss90	57 °C	200	6-FAM

Appendix 2. Mean generation time estimates for *Leptochloa crinita.*

Apéndice 2. Estimación del tiempo generacional medio en *Leptochloa crinita.*

As we did not find information on the mean generation time for *L. crinita*, we estimated a mean value of 6 years based on two lines of reasoning. In the first, we consider the following points: a) personal observations of the survival time of *L. crinita* plants in common gardens (~15 years); b) published data on survival times of other grasses in arid and semi-arid environments (~14 years, Canfield 1957; ~7 years, West et al. 1979); c) the estimation -based on points (a) and (b)- of an average survival time of 12 years [(15+14+7)/3]; d) that grasses, in general, can produce offspring from the first to the last year of their lifespan; e) that the mean generation time can be estimated by dividing the average reproductive age (6 years) by the mean number of offsprings per year (~1, assuming relatively stable populations) (Charlesworth 1994). For the second line of reasoning, we considered that grass recruitment in arid and semi-arid regions mostly occurs during rainy years (Holmgren and Scheffer 2001). We then calculated the frequency of rainy years from published literature on arid and semi-arid regions (Gray 1984; Quiroga et al. 2009; Woodhouse et al. 2010; Machado et al. 2011) finding a mean recurrence time of 6 years.

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