Title

- **Evolutionary constraints decouple genetic diversity from adaptive capacity under climate change**

Authors

Eddy Mendoza-Galindo[1,†], Francisco Molina-Freaner[2], Michiel Pillet[3,4,‡], Michelle Cabrera-Guerra[5], Antonio Hernández-López[5], Tram Vi[6], Piero Foncerrada-Elizondo[5], Diana I. Martínez-Lona[7], Tania Hernández-Hernández[4,8*]

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Other Supplementary Materials for this manuscript include the following:

Mendoza_etal2026_Supplementary Data_v1.tsv

Supplementary Methods. Health Score Determination

Health scores for each locality visited for this study were determined using the following questionnaire:

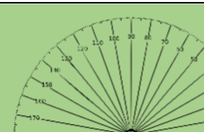
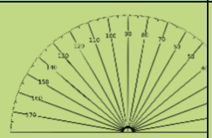
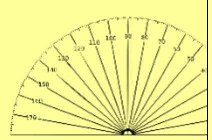
I. Abundance (number of individuals visible from a determined spot)

From a given spot in the locality, what do you see?

1. Only a handful of individuals (<5)
2. More individuals but still few, very spaced out (5-10)
3. Good number of individuals, they are closer together but still spaced out
4. Plenty of individuals, it is not possible to count them (saguaro forest)

II. Plant condition (this is the actual health of the plants, average the condition observed for all individuals in the locality; follow the next table as a guide)

1. Poor
2. Fair
3. Good
4. Excellent

	Herbivory	**Frost damage**	**Sun scorch**	**Arms**	**Dehydration**	**Leaning**	**Rot**	**Any other wound**
Excellent	None	None	None	Normal	None		None	None
Good	Little and all healed	Slight and all healed	None	Normal	None		None	Slight and healed
Fair	***Extensive but healed***	Extensive, but healed	***Orange or brown, rough epidermis***	***Slightly bent or broken***	Slight		***Healed***	***Extensive but healed***
Poor	***Not healed***	***Not healed***	***Bright yellow, smooth epidermis***	***Very bent or broken***	***Significant***		***Squishy-looking or oozing***	***Not healed***

III. Reproduction (flower/fruit/flower buds)

1. No flower buds, flowers or fruits. Flower buds look aborted
2. Extremely few flowers/fruits/flower buds, they also look small (10-20 per plant)
3. More abundant flowers/fruits/flower buds

4. High abundance of flowers/fruits/flower buds. Fruits can be found in the ground

IV. Cohort replacement (recruitment, are there babies? Young plants?)

1. No babies (>3 ft), no young plants (3-16 ft)
2. Rarely babies or young plants
3. More frequent babies or young plants, not as abundant as adults
4. High abundance of babies or young plants. As many perhaps as adult plants

*Notes: average yield for locality Sabino Canyon was estimated using average yield for Saguaro National Park E and W; which are in the same region. Yield for Superstitions locality was estimated using yield from Superior locality due to their proximity.

Supplementary Methods. DNA extraction: modified CTAB protocol

Genomic DNA was extracted from frozen tissue samples using a modified CTAB-based protocol that combined elements from two optimized procedures.
Frozen samples were thawed on ice for 10 minutes and homogenized in 750–800 µL of preheated 2× CTAB extraction buffer containing 1 M Tris-HCl (pH 8.0), 5 M NaCl, 0.25 M EDTA (pH 8.0), 0.4 mL β-mercaptoethanol, and 0.032 g PVP-40 per sample. Samples were mixed thoroughly until a uniform suspension was obtained and incubated at 55–65 °C overnight, inverting the tubes several times during incubation.
Following lysis, 4 µL RNase A (10 mg/mL) was added to each tube, and samples were incubated for an additional 30 min at 55 °C. Phase separation was performed by adding an equal volume (700–800 µL) of SEVAG solution (chloroform: isoamyl alcohol, 24 : 1 v/v), mixing gently by inversion until emulsified, and centrifuging at 14 000 rpm for 10–15 min. The upper aqueous phase was transferred to a new tube, and DNA was precipitated with 0.33 volumes of cold isopropanol (–20 °C). Samples were centrifuged at 12 000 rpm for 10 min, and the resulting pellets were air-dried at 37 °C before resuspension in 200 µL of nuclease-free water.
DNA was further purified by adding 20 µL of 2.5 M sodium acetate and 500 µL of 95% cold ethanol, incubating for 2h at –20 °C, and centrifuging at 12 000 rpm for 5 min. Pellets were washed twice with 1 mL of 70% cold ethanol, centrifuged again, briefly air-dried, and resuspended in 70 µL of nuclease-free water.
For samples requiring additional purification, the aqueous phase from the CTAB extraction was mixed with 900 µL of PB binding buffer (5 M guanidine-HCl, 30% isopropanol) and loaded onto silica spin columns pre-equilibrated with 20 µL of 2.5 M sodium acetate. Columns were washed twice with 750 µL of PE buffer (10 mM Tris-HCl, pH 7.5, 80% ethanol) and centrifuged at 14 000 rpm for 1 min each. After drying, DNA was eluted in two successive steps with 100 µL and 50 µL of pre-warmed (55 °C) TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), incubating for 15 min prior to each centrifugation.
DNA quality and yield were evaluated by spectrophotometry and agarose gel electrophoresis. Extracted DNA was stored at –20 °C for short-term use and at –80 °C for long-term preservation.

Supplementary Methods. Equations

Equation 1.

We defined the deleterious load DL as the proportion of deleterious d nonsynonymous polymorphic sites (PN) to all polymorphic sites (including synonymous polymorphic sites PS):

$$DL = PN / PS$$

Equation 2.

We manually estimated Watterson's estimator $\cup_W$ for each gene following the formula:

$$\Theta_W = \frac{S}{\sum_{i=1}^{n-1} 1/i}$$

Where S stands for the number of segregating sites (SNPs) in the gene of interest, and n the sample number of each genetic group.

Equation 3.

Tajima's D values were calculated for each gene and group using the formula:

$$D = \frac{\pi - \Theta_W}{\sqrt{Var(\pi - \Theta_W)}}$$

Equation 4.

We fitted Mixed Effects Linear Models (MELMs) as follows:

$$Health\ Score = Environmental\ variable^{i}\alpha + Genetic\ group\beta + K\mu + e$$

Where the *Health Score* is explained by the fixed effects (〈, ®) of each environmental variable *i* and the genetic group, the random effect ⎡ of the genetic kinship matrix *K*, and the residuals *e*.

Equation 5.

We used the following formula to estimate genome-wide environmental associations using MELMs:

$$y = X\alpha + P\beta + K\mu + e$$

Where y represents the observed value for each testes environmental variable, the fixed effect X represents the SNP genotype, and to correct for population structure and relatedness, we added the fixed effect $P\beta$, which represents the PCA matrix with the first two components, and the random effect of the kinship matrix $K\mu$.

Fig. S1.

Per-sample and per-site missingness rate for the unfiltered SNP data. Red dashed lines indicate thresholds used in this study.

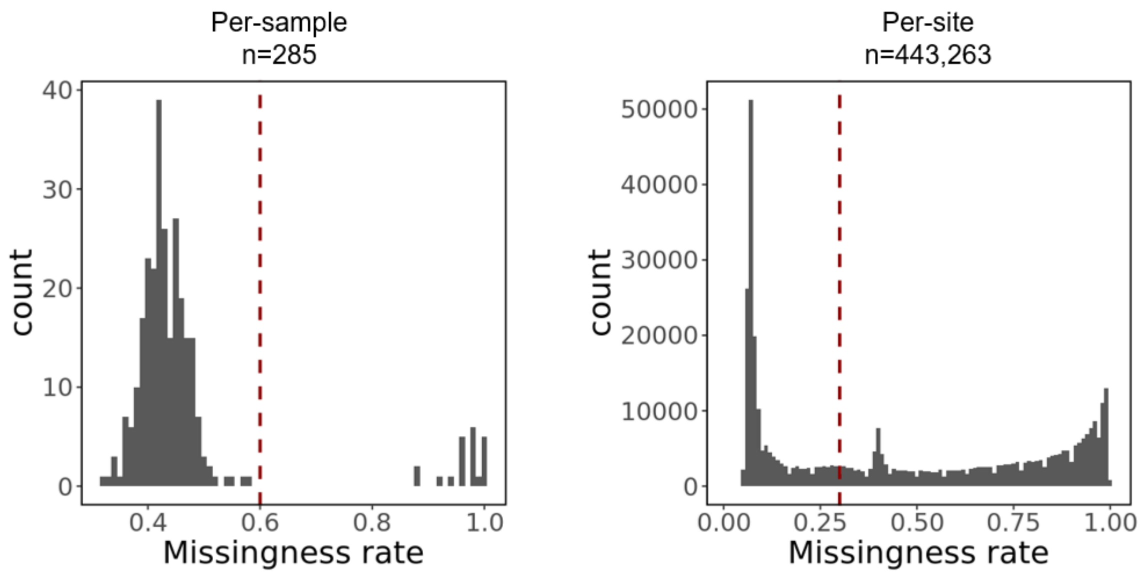


Fig. S2.
Relationship between genetic PC1 and latitude in wild saguaros. Blue dots represent the North group; green represent the South group.

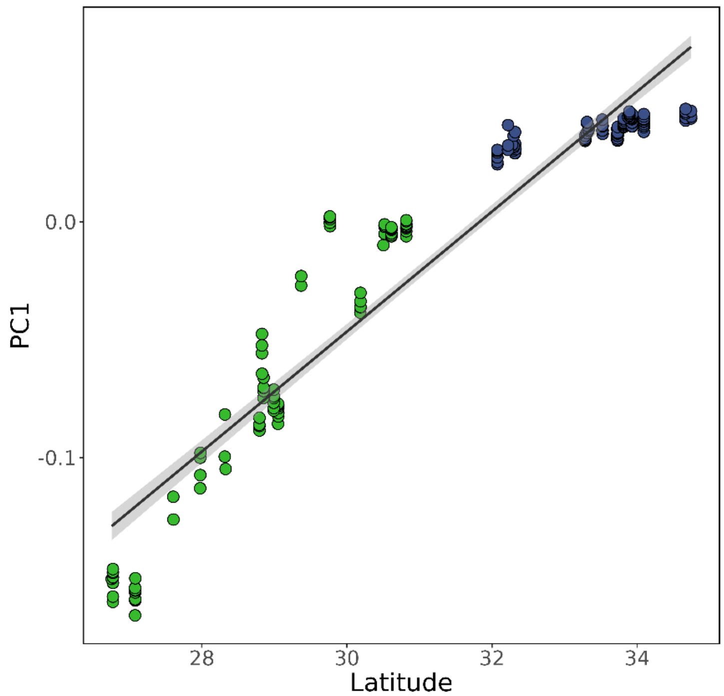


Fig. S3.
Genetic divergence between the North and South groups. Each dot represents one SNP.

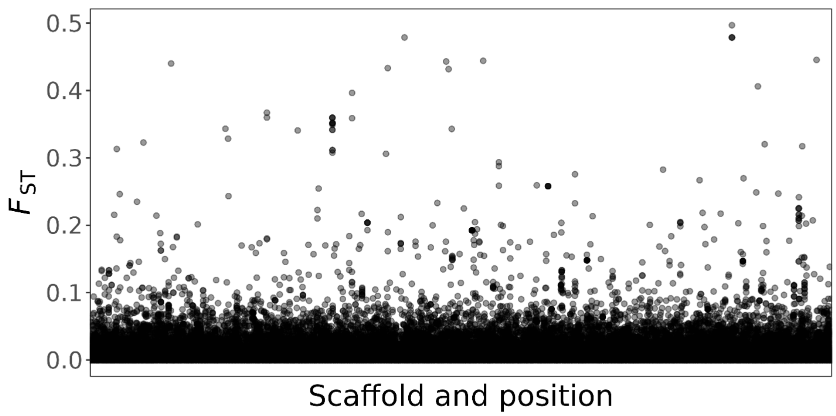


Fig. S4.

Distribution of errors in the Admixture analysis. Admixture was run with 100 random replicates per K (gray dots). Mean cross-validation error rate is shown in red for each K. Bootstrap errors were estimated by averaging the standard deviation values of ancestry coefficients per K (size of red dots). K = 5 was chosen as it shows the narrowest distribution without outliers as compared to K = 6 and K = 4.

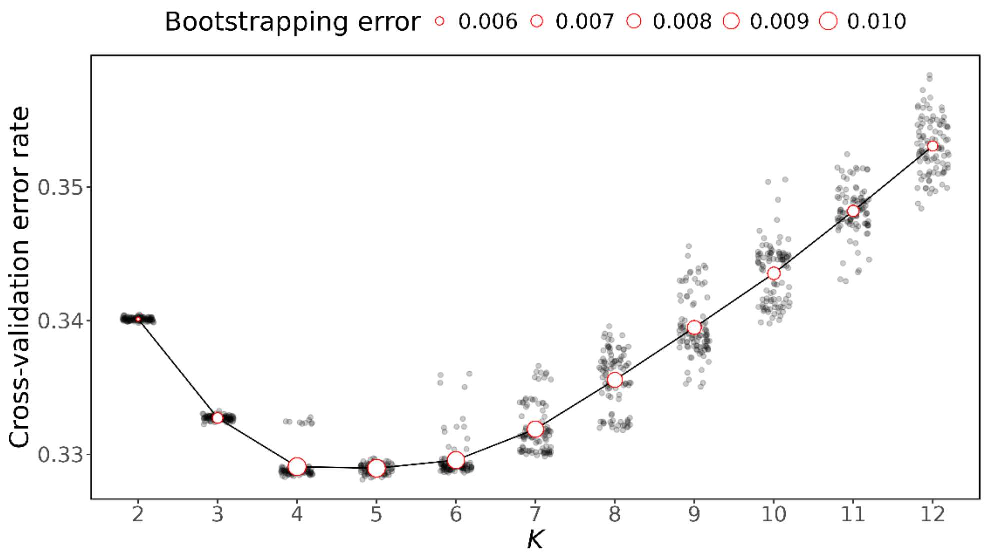


Fig. S5.
Linkage disequilibrium decay in North (blue) and South (green) groups.
Distance is calculated in base pairs. R^2 values are averaged in bins of 100 bp.

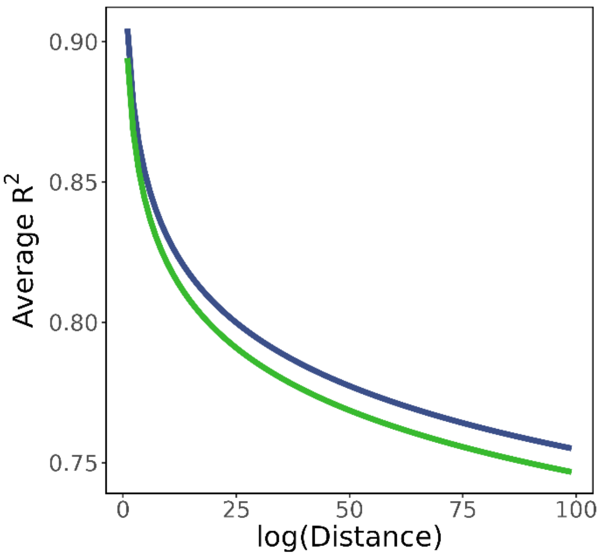


Fig. S6.
GWAS sanity checks. (A) Distribution of P values for each variable. (B) Q-Q plot for the P values.

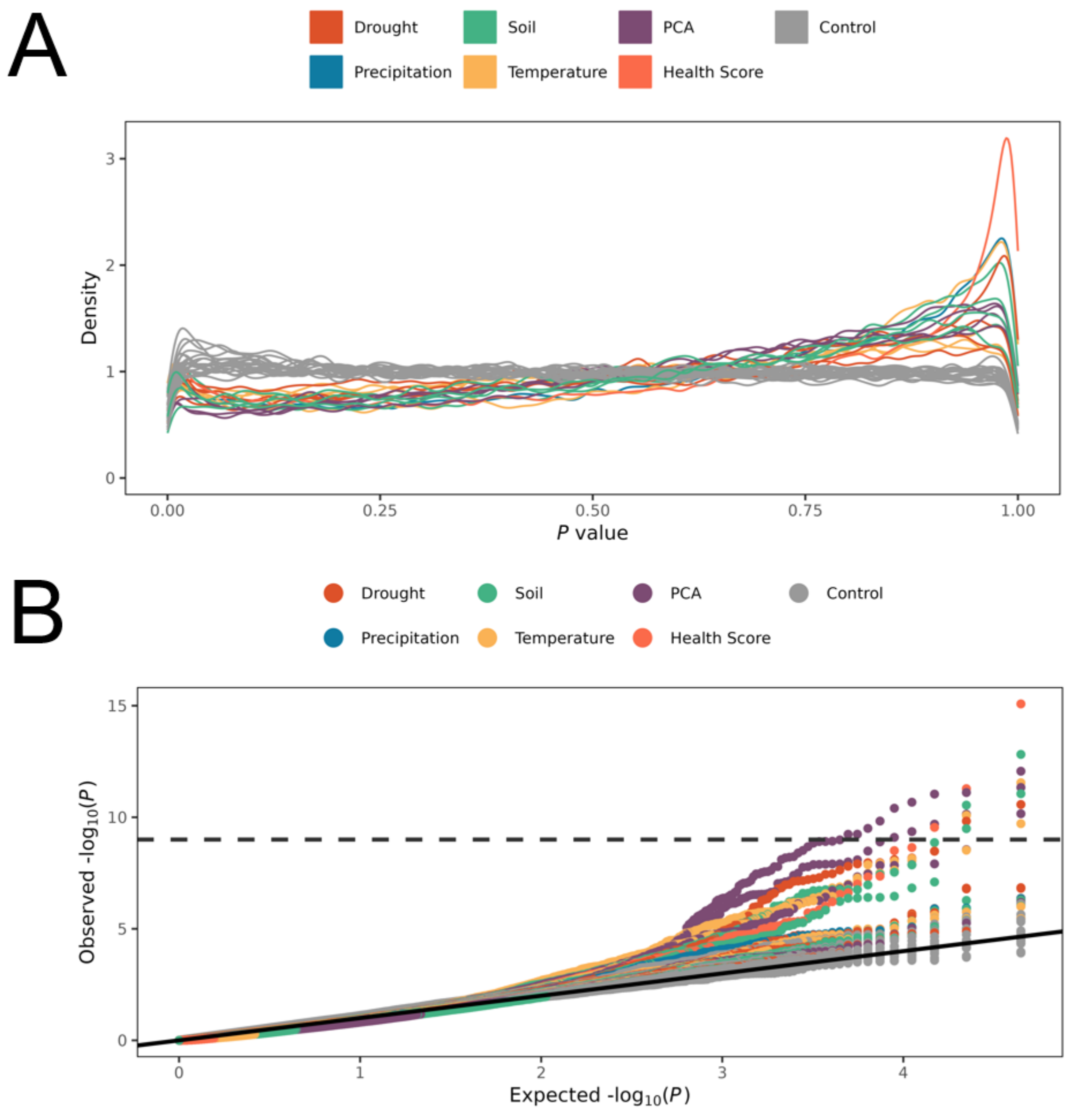


Fig. S8.
Work flow diagram illustrating the analysis of signatures of selection in candidate genes.

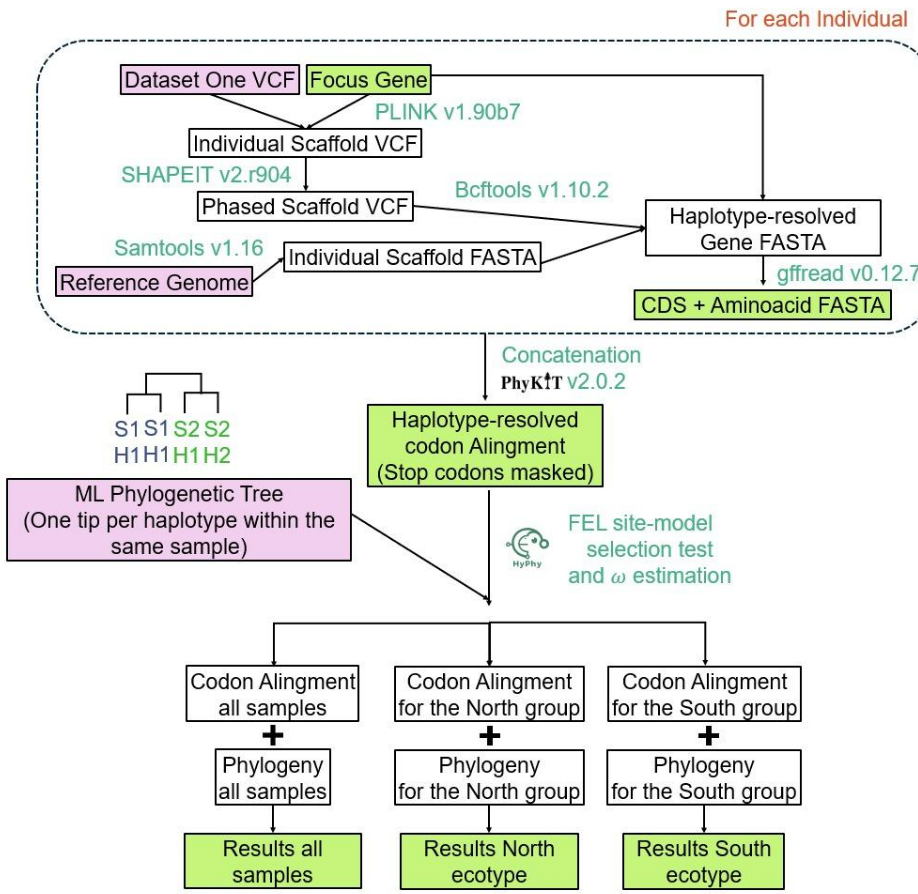


Fig. S9.

Sensitivity analysis of genomic offset using random SNP sets.
Distribution of the number of times genomic offset estimated from randomly sampled SNP sets exceeds that derived from the 17 environmentally associated candidate SNPs identified by GWAS. For each scenario, 1000 random sets of 17 SNPs were drawn from the full dataset to generate a null distribution. Vertical lines indicate the expected value under the null hypothesis (i.e., 500 out of 1000 iterations). Panels correspond to three climate scenarios: SSP1–2.6 (left), SSP3–7.0 (center), and SSP5–8.5 (right). Across all scenarios, genomic offset based on candidate adaptive SNPs consistently exceeds that expected from random SNP sets, with stronger deviations observed under higher-emission scenarios. These results support that the identified SNPs capture non-random, environmentally associated variation relevant to adaptive responses under future climate change.

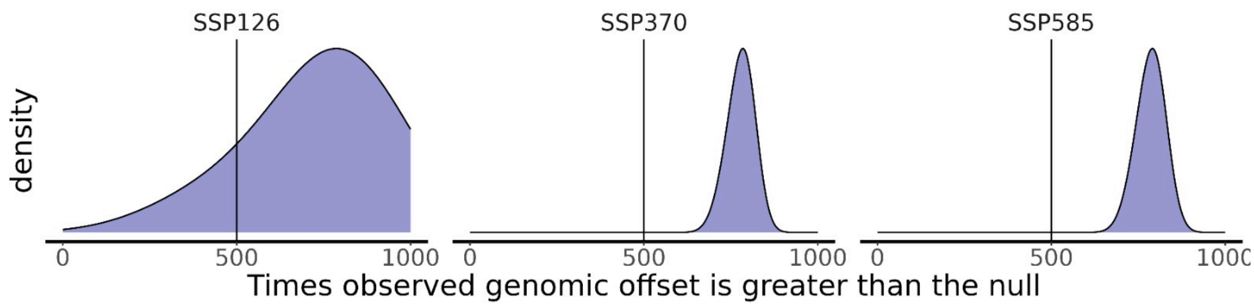


Table S1.

Media documentation of recent saguaro decline in the Phoenix Metropolitan Area.
This table summarizes representative regional, national, and international media coverage reporting increased saguaro mortality, structural failure, and physiological stress associated with extreme heat and drought in and around the Phoenix Metropolitan Area. Together, these reports illustrate growing public concern and awareness of saguaro decline and provide independent, contemporaneous documentation of the spatial extent, timing, and severity of recent impacts on this iconic desert species.

Year	Scope	Outlet	Title	Link	Description
2020	National (US)	AZ Central	Will the iconic saguaro cactus start to disappear from parts of the Southwest?	https://www.azcentral.com/story/news/local/arizona-environment/2020/06/18/saguaro-cactus-imperiled-climate-change-and-humans/3000183001/	Early warning that climate change and human pressures could push saguaros out of parts of the Sonoran Desert.
2021	Regional-Local	Desert Botanical Garden	A Disappearing Icon	https://dbg.org/a-disappearing-icon/	DBG blog noting that summer 2020 brought many reports of collapsing and failing urban saguaros around Phoenix.
2021	National (US)	The Washington Post	Saving the West's most iconic cactus from climate change	https://www.washingtonpost.com/climate-solutions/interactive/2021/saguaro-cactus-climate-change/	Drought and record heat in 2020 raised concerns about the saguaro's long-term survival under warming conditions.
2021	Regional-Local	FOX10 Phoenix	Arizonans are seeing unusual saguaro blooms…	https://www.fox10phoenix.com/news/arizonans-are-seeing-unusual-saguaro-blooms-and-experts-arent-sure-why	Experts link unusual flowering to prior heat and drought damage (2017–2020).
2023	International	Reuters	Saguaro cacti collapsing in Arizona extreme heat, scientist says	https://www.reuters.com/business/environment/saguaro-cacti-collapsing-arizona-extreme-heat-scientist-says-2023-07-25/	Global wire story linking record Phoenix heat to leaning, arm loss, and collapse.
2023	International	The Guardian	Phoenix's extreme heat withers saguaros, trademark cactus of desert landscape	https://www.theguardian.com/us-news/2023/aug/03/phoenix-extreme-heat-withers-saguaros-cactus	International coverage of saguaros failing during historic Phoenix heat.

Year	Scope	Outlet	Title	Link	Description
2023	Regional-Local	FOX10 Phoenix	Arizona saguaros are collapsing in this extreme heat, and experts are worried t	https://www.fox10phoenix.com/news/arizona-saguaros-are-collapsing-in-this-extreme-heat-and-experts-are-worried	Local reporting on urban saguaros failing during July 2023 heatwave.
2023	National (US)	CNN	Phoenix's record heat is killing off cactuses	https://www.cnn.com/2023/07/27/us/phoenix-arizona-heat-saguaro-cactuses	National coverage of cactus mortality during prolonged extreme heat.
2023	National (US)	AP News	The extreme heat in Phoenix is withering some of its famed saguaro cactuses, with no end in sight	https://apnews.com/article/heat-wave-phoenix-cactus-plants-974dfd0a5b19f804837d90a96b567ede	Residents and DBG share images of damaged and collapsed saguaros.
2023	Regional-Local (AZ)	Desert Diaries (Desert Museum)	Are saguaros dying in Arizona?	https://blog.desertmuseum.org/2023/08/30/are-saguaros-dying-in-arizona/	Explains mechanisms such as night heat and stem softening behind collapses.
2023	Regional-Local (AZ)	Axios Phoenix	Help save cactuses with the 2023 saguaro census	https://www.axios.com/local/phoenix/2023/05/08/saguaro-cactus-census-2023-phoenix-desert-botanical-garden	Launch of community census following reports of widespread saguaro decline.
2023	International	12News	Los saguaros urbanos caen muertos debido al calor extremo …	https://www.12news.com/article/syndication/spanish/los-saguaros-urbanos-estan-cayendo-muertos-por-el-calor-extremo-y-nosotros-le-mostramos-como-salvarlos-en-su-propiedad/75-7ed2eff2-3f86-462b-be96-cf5482ebc374	Spanish-language explainer on urban saguaros collapsing in Phoenix.
2023	International	Univision	Ola de calor excesivo está acabando con los saguaros	https://www.univision.com/local/arizona-ktvw/ola-de-calor-pone-en-riesgo-saguaros-arizona-fotos	Photo report on saguaros collapsing due to extreme heat and lack of rain.
2023	International	Yahoo Noticias (ES)	Calor extremo en Phoenix también estresa a saguaros, árboles frutales y otras plantas	https://es-us.noticias.yahoo.com/calor-extremo-phoenix-estresa-saguaros-011321043.html	Spanish language article reports on how intense heat waves in Phoenix are stressing and damaging saguaro cacti, causing many to weaken and even die in the metropolitan area.

Year	Scope	Outlet	Title	Link	Description
2023	International	Le HuffPost	Même ces cactus mythiques ne supportent plus la chaleur	https://www.huffingtonpost.fr/international/video/en-arizona-meme-ces-cactus-mythiques-ne-supportent-plus-les-conditions-climatiques-extremes_221608.html	French video/article on saguaros failing under extreme conditions.
2023	International	TF1 INFO	États-Unis : face à la chaleur en Arizona, même les cactus s'effondren	https://www.tf1info.fr/environnement-ecologie/etats-unis-changement-climatique-face-a-la-vague-de-chaleur-canicule-a-phoenix-en-arizona-meme-les-cactus-s-effondrent-2266351.html	French TV site: Phoenix street saguaros losing arms/collapsing.
2023	International	RND.de	Rekordhitze in Arizona: Meterhohe Saguaro-Kakteen kippen einfach um – „wie gebacken	https://www.rnd.de/panorama/arizona-rekord-hitze-laesst-saguaro-kakteen-umkippen-PTWTQHRWQ5JONHG6SAIMBYOOAE.html	German national outlet reporting saguaros toppling during record heat.
2023	International	ABC news	VIDEO: Arizona's extreme heat and drought is impacting the state's iconic Saguaro	https://www.abc.net.au/news/2023-07-26/arizonas-extreme-heat-and-drought-is-impacting-the-saguaro/102650164	A report in DBG states that the saguaro cacti are suffering from the intense heat and drought.
2024	Regional-Local (AZ)	AZCentral	Heat, extreme weather can kill Arizona’s saguaros	https://www.azcentral.com/story/news/local/arizona-environment/2024/03/18/arizona-saguaros-desert-plants-dying-as-climate-changes/72843966007/	Explainer linking saguaros’ decline to heat, drought, and infection risk.
2024	Regional-Local (AZ)	OPB	Giant old saguaros can be resilient. It’s baby saguaros researchers are worried about	https://www.opb.org/article/2024/08/14/giant-old-saguaros-can-be-resilient-it-s-baby-saguaros-researchers-are-worried-about/	The young seedlings are more vulnerable to extreme conditions, which could affect the future regeneration of the species.
2024	Regional-Local (AZ)	KPBS	Giant old saguaros can be resilient. It’s baby saguaros researchers are worried about	https://www.opb.org/article/2024/08/14/giant-old-saguaros-can-be-resilient-it-s-baby-saguaros-researchers-are-worried-about/	The young seedlings are more vulnerable to extreme conditions, which could affect the future regeneration of the species.

Year	Scope	Outlet	Title	Link	Description
2024	Regional-Local (AZ)	Arizona's Family	Are the recent hot summers stressing out Phoenix-based saguaros?	https://www.azfamily.com/2024/09/12/are-recent-hot-summers-stressing-out-phoenix-based-saguaros/	Local segment citing DBG figures and heat/drought linkage.
2025	National (US)	CBS News	Arizona's iconic saguaro cactus was built for desert heat. Rising temperatures are pushing it to the brin**k**	https://www.cbsnews.com/news/arizona-saguaro-cactus-built-desert-heat-rising-temperatures-pushing-to-brink/	National broadcast reporting rising saguaro mortality in Arizona.
2025	National (US)	Axios Phoenix	Scorching saguaros: Climate extremes are killing cactuses	https://www.axios.com/local/phoenix/2025/05/19/scorching-saguaros-heat-drought-cactus-deaths	DBG notes tracked ~1,000 saguaros; stress and mortality increased after 2021
2025	Regional-Local (AZ)	ABC15 Arizona in Phoenix (KNXV)	Arizona's most resilient desert species facing pressure amid the state's hotter summers	https://www.abc15.com/weather/impact-earth/arizonas-most-resilient-desert-species-facing-pressure-amid-the-states-hotter-summers	DBG say saguaro mortality rates have jumped to nearly 7 percent in recent years,
2025	International	CBS Evening News (YouTube)	The desert is getting too hot for the saguaro cactus	https://www.youtube.com/watch?v=2p2Zf22PE3E	Global segment on saguaros collapsing during multi-week extreme heat events.
2025	Regional-Local (AZ)	Arizona's Family	Arizona's extreme heat threatens iconic saguaro cactus, researchers say	https://www.azfamily.com/2025/05/24/arizonas-extreme-heat-threatens-iconic-saguaro-cactus-researchers-say/	Researchers warn that extreme heat is stressing the saguaro cacti, causing disease and a near absence of seedlings
2025	Regional-Local (AZ)	Rosie on the House	The Mighty Saguaro, Radio interview	https://rosieonthehouse.com/outdoor-living-hour-saguaros-with-dr-tania-hernandez-of-the-arizona-botanical-garden-and-author-barbara-bash/	DBG encourages citizens to join the saguaro protection project.

Table S2.
Population genetics summary statistics from 46,195 genome-wide SNPs.

Group			*n*	Observed Heterozygosity	Expected Heterozygosity	Inbreeding coefficient (FIS)	Nucleotide diversity (π)	Genome-wide Tajima's D	Percentage of segregating sites	Percentage of private sites	Deleterious load (x100)
All samples			248	0.1603	0.1907	0.1616	0.1911	0.8519	100	0	0.913±0.630
South	All		94	**0.1635**	**0.1953**	**0.1685**	**0.1964**	**0.4596**	**83.4711**	**2.1494**	**1.00±0.681**
North	All*		154	**0.1583**	**0.1856**	**0.1503**	**0.1862**	**0.5549**	**98.6622**	**0**	**0.857±0.593**
	Wild		107	0.1554	0.1688	0.0838	0.1696	1.1303	62.8831	2.0938	0.826±0.566
	Ex-situ	DBG	23	0.1604	0.1905	0.181	0.195	0.2115	79.2819	0.2709	0.983±0.726
		Urban	24	0.1694	0.192	0.1402	0.1963	0.0652	83.1059	0.8411	0.875±0.579

*Although the North-All group includes individuals currently maintained in ex situ collections, these samples derive from the North lineage, as saguaros are not artificially cultivated and thus reflect their original wild provenance.

Table S3.
SNP datasets and filtering details.

Name of dataset	**VCF-1**	**VCF-2**	**VCF-3**	**VCF-4**
Analyses	**Population structure**	**Genetic diversity and differentiation**	**Wild saguaro genetics**	**Demographic inference**
Source	Stacks output	Stacks output	VCF-1	VCF-1
Sample group	All individuals (including technical duplicates)	Wild and *ex situ* individuals (duplicated and odd samples removed)	Wild individuals only	SameasVCF-2
Number of individuals	261	248	201	248
Minimum genotype depth	4	4	No new filter used	No new filter used
Maximum genotype depth	250	250	No new filter used	No new filter used
Maximum missing per individual	0.6	0.6	No new filter used	No new filter used
Maximum missing per locus	0.3	0.3	0.3	0.3
MAF	0.01	0.01	0.01	0.01
LD	$r^2 \geq 0.1$	No filter used	No filter used	$r^2 \geq 0.1$
Number of SNPs	19,280 (46,647 without this filter)	46,195	44,711	19,000
Used for	PCA, Admixture, ML Tree and UPGMA	AMOVA, snpR stats, PiXY gene analysis, selection analysis	Genetic differentiation, GWAS, RDA	Demographic inference
VCFmd5sum	eff0e56cc419e0805a99ebb b8c014398	b70579ca1ada0d95ff51747 9602e94e2	9e235d956f6151db2a4da1 93d2b79225	c72ab588e23751ba849abff2 54becb54

Table S4.
Candidate genes under divergent selection between North and South saguaro lineages.
These genes were identified as those with $\Delta\pi > 0.2$, $F\_ST > 0.25$, and opposite Tajima's D signs among North and South lineages.

GeneID	Δπ (S–N)	FST	Tajima's D (N/S)	Focus lineage	Description / Functional annotation
Cgig2_019831	0.388	0.262	1.74/–1.71	North	Unknown function
Cgig2_017565	0.353	0.305	2.78/–0.07	North	Unknown function
Cgig2_023402	0.260	0.254	–1.48/0.42	South	TRAFAC class myosin-kinesin ATPase superfamily protein
Cgig2_012159	0.271	0.268	–0.43/1.88	South	CRISP family protein
Cgig2_011450	0.280	0.361	–0.39/1.99	South	Alpha beta-hydrolase superfamily protein
Cgig2_024234	0.285	0.329	–0.73/1.61	South	External alternative NAD(P)H-ubiquinone oxidoreductase
Cgig2_000565	0.288	0.323	–0.72/1.65	South	Unknown function
Cgig2_022772	0.301	0.415	–0.13/2.48	South	Pentatricopeptide repeat-containing protein (PPR)
Cgig2_033157	0.301	0.348	–0.39/2.15	South	Plant lipid-transfer protein (LTP) family
Cgig2_007705	0.324	0.254	–0.01/2.78	South	Conserved protein UCP012943
Cgig2_006466	0.329	0.268	–1.09/1.56	South	AT-hook DNA-binding protein
Cgig2_019515	0.330	0.251	–0.37/2.45	South	Transcription factor TFIID (TATA-binding protein)
Cgig2_030266	0.343	0.329	–1.26/1.45	South	Protein folding chaperone (ATP hydrolysis-dependent)
Cgig2_016797	0.357	0.292	–0.46/2.51	South	Unknown function
Cgig2_016798	0.357	0.292	–0.46/2.51	South	Unknown function (duplicate locus)
Cgig2_019070	0.379	0.322	–0.09/3.20	South	Unknown function
Cgig2_001666	0.381	0.303	–0.23/3.07	South	Helicase protein
Cgig2_022771	0.386	0.443	–0.45/2.85	South	Unknown function
Cgig2_027032	0.390	0.309	–0.31/3.03	South	Hydroxyproline-rich glycoprotein family protein
Cgig2_008678	0.410	0.418	–0.95/2.46	South	Vesicle transport protein
Cgig2_022770	0.463	0.455	–0.45/3.52	South	Heat-shock protein 90
Cgig2_033583	0.500	0.588	–0.28/4.07	South	Unknown function
Cgig2_026828	–0.451	0.311	3.48/–0.16	North	Vesicle transport protein
Cgig2_032487	–0.299	0.272	1.15/–1.64	North	Unknown function
Cgig2_032488	–0.299	0.272	1.15/–1.64	North	Unknown function (duplicate window)
Cgig2_031306	0.271	0.259	–0.02/2.28	South	Phosphate transporter PHO1 homolog
Cgig2_032295	0.276	0.282	–0.63/1.64	South	Protein FANTASTIC FOUR
Cgig2_015686	0.276	0.400	–0.03/2.37	South	Unknown function
Cgig2_012415	0.298	0.254	–0.06/2.54	South	LRR receptor-like serine/threonine kinase
Cgig2_014336	0.301	0.278	–0.20/2.41	South	Plant mobile domain protein
Cgig2_020446	0.303	0.285	–0.28/2.33	South	Unknown function
Cgig2_012270	0.314	0.439	–0.12/2.59	South	Vacuolar fusion protein CCZ1 homolog
Cgig2_016300	0.314	0.335	–0.26/2.45	South	Type III restriction enzyme (res subunit)
Cgig2_013878	0.335	0.255	–0.43/2.44	South	Eukaryotic translation initiation factor isoform

Cgig2_030267	0.344	0.253	–0.07/2.90	South	Prohibitin-3
Cgig2_020395	0.355	0.260	–0.55/2.43	South	50S ribosomal protein 5
Cgig2_033197	0.415	0.345	–0.27/3.32	South	Clp protease-related protein (At4g12060 homolog)
Cgig2_002414	0.434	0.375	–0.28/3.49	South	Unknown function
Cgig2_029364	0.435	0.433	–0.40/3.31	South	AWPM-19-like membrane family protein
Cgig2_010757	0.457	0.424	–0.32/3.63	South	Importin-9
Cgig2_012216	0.463	0.463	–0.13/3.91	South	HNH endonuclease

Table S5.
Association tests between Health-Score and the environmental ordination (PCA). Linear mixed effects models were set to control for genetic group (Q) and kinship (K). P values correspond to Type II Wald Chi-square tests.

Variable	**Variable category**	**Correlation value (R)**	**FDR-adjusted P value**
hms_median	Drought	-0.11820905	1.52E-03
had_third_quartile	Drought	0.10646657	2.18E-02
hms_third_quartile	Drought	-0.09600398	1.14E-01
had_mean	Drought	0.04935119	9.87E-01
env_PC1	PCA	-0.04271489	3.10E-12
env_PC2	PCA	0.20470545	3.76E-02
bio14	Precipitation	0.29763945	1.39E-02
cfvo	Soil	0.41523945	1.72E-06
cec	Soil	0.19598772	1.39E-03
ph	Soil	-0.29357457	5.43E-03
soc	Soil	0.10508791	1.05E-01
sand	Soil	-0.13448592	3.34E-01
bio5	Temperature	-0.3193824	2.07E-07
bio8	Temperature	0.21039274	3.33E-06
bio9	Temperature	-0.27510541	5.82E-04
bio2	Temperature	-0.18351356	5.92E-03

Table S6.

Environmental differentiation tests between North and South populations for the current climatic scenario. Twenty-five uncorrelated variables (r > 0.90) are shown. FDR-corrected P values correspond to ANOVA Type II tests. P values were corrected for multiple testing using the Benjamini–Hochberg False Discovery Rate (FDR). Effect sizes were calculated using Cohen's method.

Variable	Variable category	FDR-adjusted P value	Effect size
env_PC1_no_drought	PCA	1.86E-45	2.7036
env_PC1	PCA	8.60E-45	2.6611
bio15	Precipitation	5.12E-43	2.5652
bio7	Temperature	2.68E-41	2.4778
silt	Soil	1.28E-30	1.9733
hms_median	Drought	6.92E-24	1.6594
bio13	Precipitation	9.20E-23	1.6045
bio8	Temperature	3.80E-14	1.1775
sand	Soil	2.34E-13	1.1338
bio5	Temperature	5.33E-12	1.0596
hms_mean	Drought	2.31E-11	1.0234
bio12	Precipitation	5.62E-11	1.0007
bio19	Precipitation	2.31E-10	0.9644
bio14	Precipitation	8.11E-09	0.8708
bio10	Temperature	6.08E-08	0.8145
bio1	Temperature	9.48E-08	0.801
ph	Soil	2.44E-05	0.6266
bio17	Precipitation	4.05E-05	0.6082
had_mean	Drought	6.83E-04	0.5035
had_third_quartile	Drought	1.68E-03	0.466
cfvo	Soil	1.17E-02	0.373
env_PC2	PCA	1.84E-02	0.3489
bio9	Temperature	5.71E-02	0.2846
bio2	Temperature	9.02E-02	0.2544
soc	Soil	3.89E-01	0.1357
cec	Soil	4.24E-01	0.1225
hms_third_quartile	Drought	4.40E-01	0.1178
env_PC2_no_drought	PCA	9.64E-01	0.0133
clay	Soil	9.86E-01	0.0024

TableS7.
Marginal genetic variance explained by individual RDA predictors.

Variable	Genetic variance explained (%)	P value
bio14	1.79	0.000999001
bio8	1.19	0.000999001
soc	1.1	0.000999001
ph	1.06	0.000999001
had_third_quartile	0.97	0.001998002
bio9	0.95	0.000999001
bio5	0.93	0.000999001
had_mean	0.93	0.001998002
bio2	0.92	0.000999001
cec	0.88	0.000999001
hms_third_quartile	0.81	0.000999001
sand	0.79	0.001998002
cfvo	0.69	0.003996004
hms_median	0.67	0.015984016

Table S8a.

Candidate SNP-environment associations. Candidate adaptive single-nucleotide polymorphisms (SNPs) significantly associated with environmental variables and environmental ordination axes identified through genotype-environment association analyses. For each SNP, the table reports the raw P value, false discovery rate (FDR), sample size (n), associated environmental predictor, predictor category, genomic location, and nearest annotated gene.

SNP	P value	FDR	Env. predictor	n	Category	Chromosome	Position	Associated gene	Locus
JAKOGI010005138.1_3137	2.67E-11	1.19E-06	hms_third_quartile	192	Drought	JAKOGI010005138.1	3137	Cgig2_014344	JAKOGI010005138.1:1272-4151
JAKOGI010000002.1_1708068	8.24E-11	1.84E-06	bio8	192	Temperature	JAKOGI010000002.1	1708068	Cgig2_014691	JAKOGI010000002.1:1695261-1711118
JAKOGI010000270.1_751529	2.82E-12	1.26E-07	bio8	189	Temperature	JAKOGI010000270.1	751529	Cgig2_025494	JAKOGI010000270.1:744284-769660
JAKOGI010000729.1_361226	2.86E-11	6.37E-07	sand	201	Soil	JAKOGI010000729.1	361226	Cgig2_022984	JAKOGI010000729.1:336609-363267
JAKOGI010000134.1_249246	8.80E-12	3.92E-07	sand	198	Soil	JAKOGI010000134.1	249246	Cgig2_011008	JAKOGI010000134.1:243547-249110
JAKOGI010000008.1_307729	7.80E-12	1.36E-07	PC2	176	PCA	JAKOGI010000008.1	307729	Cgig2_018149	JAKOGI010000008.1:306175-310878
JAKOGI010000156.1_269330	4.63E-12	1.36E-07	PC2	145	PCA	JAKOGI010000156.1	269330	Cgig2_019061	JAKOGI010000156.1:268594-275239
JAKOGI010000822.1_314674	5.79E-10	2.87E-06	PC2	161	PCA	JAKOGI010000822.1	314674	Cgig2_007096	JAKOGI010000822.1:312713-320794
JAKOGI010001073.1_55981	3.15E-10	2.01E-06	PC2	184	PCA	JAKOGI010001073.1	55981	Cgig2_023240	JAKOGI010001073.1:53822-59525
JAKOGI010003692.1_33249	5.74E-10	2.87E-06	PC2	176	PCA	JAKOGI010003692.1	33249	Cgig2_012478	JAKOGI010003692.1:33260-43311

Table S8b.

Functional information of genes reported in Table 8a. For each SNP, the associated gene, genomic annotation, full Gene Ontology (GO) term identifiers, condensed functional interpretation of GO categories, and PFAM protein domains are reported when available.

SNP	Associated gene	Description	GOs (full list)	Condensed functional label	PFAMs
JAKOGI010005138.1_3137	Cgig2_014344	Unknown	Unknown	Unknown	Unknown
JAKOGI010000002.1_1708068	Cgig2_014691	Methylmalonate-semialdehyde dehydrogenase	GO:0003674,GO:0005488,GO:0005507,GO:0005575,GO:0005622,GO:0005623,GO:0005737,GO:0005739,GO:0006950,GO:0006979,GO:0008150,GO:0043167,GO:0043169,GO:0043226,GO:0043227,GO:0043229,GO:0043231,GO:0044424,GO:0044444,GO:0044464,GO:0046872,GO:0046914,GO:0050896	Metabolic regulation and stress response	Aldedh
JAKOGI010000270.1_751529	Cgig2_025494	Actin filament capping and severing protein	GO:0001101,GO:0003674,GO:0003779,GO:0005488,GO:0005515,GO:0005575,GO:0005622,GO:0005623,GO:0005737,GO:0005829,GO:0005856,GO:0006810,GO:0006996,GO:0007010,GO:0007015,GO:0007275,GO:0008064,GO:0008092,GO:0008150,GO:0008154,GO:0009653,GO:0009719,GO:0009725,GO:0009737,GO:0009888,GO:0009987,GO:0010015,GO:0010033,GO:0010035,GO:0010038,GO:0010053,GO:0010054,GO:0010639,GO:0015629,GO:0016043,GO:0016049,GO:0016482,GO:0021700,GO:0022411,GO:0022622,GO:0030029,GO:0030036,GO:0030042,GO:0030154,GO:0030832,GO:0030833,GO:0030834,GO:0030835,GO:0030837,GO:0031333,GO:0032271,GO:0032272,GO:0032432,GO:0032501,GO:0032502,GO:0032535,GO:0032956,GO:0032970,GO:0032984,GO:0033043,GO:0033993,GO:0040007,GO:0042221,GO:0043226,GO:0043228,GO:0043229,GO:0043232,GO:0043242,GO:0043244,GO:0043254,GO:0043624,GO:0043933,GO:0044087,GO:0044422,GO:0044424,GO:0044430,GO:0044444,GO:0044446,GO:0044464,GO:0044877,GO:0046907,GO:0048364,GO:0048468,GO:0048469,GO:0048519,GO:0048523,GO:0048588,GO:0048589,GO:0048731,GO:0048764,GO:0048765,GO:0048767,GO:0048856,GO:0	Cytoskeleton organization and stress response	Gelsolin,VHP

			048869,GO:0050789,GO:0050794,GO:0050896,GO:0051014,GO:0051015,GO:0051128,GO:0051129,GO:0051179,GO:0051234,GO:0051261,GO:0051493,GO:0051494,GO:0051592,GO:0051641,GO:0051649,GO:0051693,GO:0060560,GO:0065007,GO:0065008,GO:0071695,GO:0071840,GO:0080147,GO:0090066,GO:0090558,GO:0090627,GO:0097305,GO:0097435,GO:0099402,GO:0099636,GO:0110053,GO:1901700,GO:1901879,GO:1901880,GO:1902903,GO:1902904,GO:1905392		
JAKOGI010000729.1_361226	Cgig2_022984	Protein of unknown function (DUF810)	GO:0005575,GO:0005622,GO:0005623,GO:0005737,GO:0005886,GO:0005911,GO:0008150,GO:0009506,GO:0009987,GO:0010118,GO:0016020,GO:0030054,GO:0044424,GO:0044464,GO:0055044,GO:0071944	Membrane-associated protein, unknown function	DUF810
JAKOGI010000134.1_249246	Cgig2_011008	E3ubiquitin-protein ligase	GO:0000209,GO:0003674,GO:0003824,GO:0004842,GO:0005575,GO:0006464,GO:0006508,GO:0006511,GO:0006807,GO:0007275,GO:0008150,GO:0008152,GO:0009056,GO:0009057,GO:0009553,GO:0009555,GO:0009561,GO:0009987,GO:0010498,GO:0016020,GO:0016567,GO:0016740,GO:0019538,GO:0019787,GO:0019941,GO:0030163,GO:0032446,GO:0032501,GO:0032502,GO:0036211,GO:0043161,GO:0043170,GO:0043412,GO:0043632,GO:0044237,GO:0044238,GO:0044248,GO:0044257,GO:0044260,GO:0044265,GO:0044267,GO:0048229,GO:0048856,GO:0050789,GO:0050794,GO:0051603,GO:0051726,GO:0055046,GO:0061630,GO:0061659,GO:0065007,GO:0070647,GO:0071704,GO:0140096,GO:1901564,GO:1901565,GO:1901575	Protein turnover and stress signaling	zf-C3HC4_2,zf-RING_2,zf-rbx1
JAKOGI010000008.1_307729	Cgig2_018149	Squamosa promoter-binding-like protein	GO:0003674,GO:0003676,GO:0003677,GO:0003700,GO:0005488,GO:0005575,GO:0005622,GO:0005623,GO:0005634,GO:0006355,GO:0007275,GO:0008150,GO:0009653,GO:0009889,GO:0009891,GO:0009893,GO:0009965,GO:0010016,GO:0010358,GO:0010468,GO:0010556,GO:0010557,GO:0010604,GO:0010628,GO:0019219,GO:0019222,GO:0031323,GO:0031325,GO:0031326,GO:0031328,GO:0032501,GO:0032502,GO:0040034,GO:0043226,GO:0043227,GO:0043229,GO:0043231,GO:0043565,GO:004442	Transcriptional regulation and development	SBP

			4,GO:0044464,GO:0045893,GO:0045935,GO:0048366,GO:0048367,GO:0048506,GO:0048509,GO:0048510,GO:0048518,GO:0048522,GO:0048532,GO:0048731,GO:0048827,GO:0048856,GO:0050789,GO:0050793,GO:0050794,GO:0051171,GO:0051173,GO:0051252,GO:0051254,GO:0060255,GO:0065007,GO:0080090,GO:0097159,GO:0099402,GO:0140110,GO:1901363,GO:1902680,GO:1903506,GO:1903508,GO:1905392,GO:2000112,GO:2001141		
JAKOGI010000156.1_269330	Cgig2_019061	Pseudouridinesynthase	Unknown	RNA modification and translational regulation	TruB_C_2,TruB_N
JAKOGI010000822.1_314674	Cgig2_007096	Nucleobase–ascorbate transporter	GO:0003674,GO:0005215,GO:0005345,GO:0005350,GO:0005575,GO:0005623,GO:0005886,GO:0006810,GO:0006811,GO:0006812,GO:0006855,GO:0006863,GO:0008150,GO:0008324,GO:0015075,GO:0015205,GO:0015207,GO:0015208,GO:0015210,GO:0015238,GO:0015291,GO:0015293,GO:0015294,GO:0015318,GO:0015851,GO:0015853,GO:0015854,GO:0015855,GO:0015857,GO:0015893,GO:0016020,GO:0022804,GO:0022857,GO:0022890,GO:0034220,GO:0035344,GO:0042221,GO:0042493,GO:0044464,GO:0050896,GO:0051179,GO:0051234,GO:0055085,GO:0071702,GO:0071705,GO:0071944,GO:0072530,GO:0072531,GO:0098655,GO:0098657,GO:0098702,GO:0098710,GO:0098721,GO:0098739,GO:1903716,GO:1903791,GO:1904082,GO:1904823	Membrane transport and nutrient exchange	Xan_ur_permease
JAKOGI010001073.1_55981	Cgig2_023240	Sec-independent protein translocase subunitTatB	GO:0003674,GO:0005215,GO:0005575,GO:0005622,GO:0005623,GO:0005737,GO:0005886,GO:0006605,GO:0006612,GO:0006810,GO:0006886,GO:0006950,GO:0008104,GO:0008150,GO:0008320,GO:0008565,GO:0009266,GO:0009409,GO:0009507,GO:0009526,GO:0009534,GO:0009535,GO:0009536,GO:0009579,GO:0009628,GO:0009889,GO:0009941,GO:0009977,GO:0010119,GO:0015031,GO:0015291,GO:0015399,GO:0015405,GO:0015450,GO:0015833,GO:0016020,GO:0017038,GO:0019222,GO:0022804,GO:0022857,GO:0022884,GO:0031323,GO:0031967,GO:0031975,GO:0031976,GO:0031984,GO:0032991,GO:0033036,GO:0033281,GO:0033365,GO:003	Protein translocation and stress response	MttA_Hcf106

			4357,GO:0034613,GO:0042651,GO:0042886,GO:0042887,GO:0043226,GO:0043227,GO:0043229,GO:0043231,GO:0043953,GO:0044422,GO:0044424,GO:0044425,GO:0044434,GO:0044435,GO:0044436,GO:0044444,GO:0044446,GO:0044459,GO:0044464,GO:0044743,GO:0045036,GO:0045038,GO:0045184,GO:0046907,GO:0048518,GO:0048522,GO:0048583,GO:0050789,GO:0050794,GO:0050896,GO:0051179,GO:0051234,GO:0051641,GO:0051649,GO:0055035,GO:0055085,GO:0065002,GO:0065007,GO:0070727,GO:0071702,GO:0071705,GO:0071806,GO:0071944,GO:0072594,GO:0072596,GO:0072598,GO:0072657,GO:0080134,GO:0090150,GO:0098796,GO:0098797,GO:1902456,GO:1902458,GO:1903426,GO:1904680,GO:2000070,GO:2000377		
JAKOGI010003692.1_33249	Cgig2_012478	Pentatricopeptide repeat-containing protein	GO:0003674,GO:0003676,GO:0003723,GO:0003824,GO:0004518,GO:0004519,GO:0005488,GO:0005575,GO:0005622,GO:0005623,GO:0006139,GO:0006725,GO:0006807,GO:0008150,GO:0008152,GO:0009451,GO:0009987,GO:0016070,GO:0016787,GO:0016788,GO:0034641,GO:0043170,GO:0043226,GO:0043227,GO:0043229,GO:0043231,GO:0043412,GO:0044237,GO:0044238,GO:0044424,GO:0044464,GO:0046483,GO:0071704,GO:0090304,GO:0090305,GO:0097159,GO:1901360,GO:1901363	Organelle gene regulation and stress response	PPR,PPR_1,PPR_2,PPR_3

Table S9.
ANOVA for the effect of the genetic group on the genomic offset.

Fixed Effect	Chi-squared value	Degrees of Freedom	P value
Genetic group	39.19386	1	3.83e-10
Scenario (SSP)	9142.90443	2	0
Geneticgroup X Scenario	38.8483	2	3.66e-09