

CONSERVATION GENETICS

Individual heterozygosity predicts translocation success in threatened desert tortoises

Peter A. Scott^{1,2*}, Linda J. Allison³, Kimberleigh J. Field³, Roy C. Averill-Murray³, H. Bradley Shaffer^{1,4}

Anthropogenic environmental modification is placing as many as 1 million species at risk of extinction. One management action for reducing extinction risk is translocation of individuals to locations from which they have disappeared or to new locations where biologists hypothesize they have a good chance of surviving. To maximize this survival probability, the standard practice is to move animals from the closest possible populations that contain presumably related individuals. In an empirical test of this conventional wisdom, we analyzed a genomic dataset for 166 translocated desert tortoises (*Gopherus agassizii*) that either survived or died over a period of two decades. We used genomic data to infer the geographic origin of translocated tortoises and found that individual heterozygosity predicted tortoise survival, whereas translocation distance or geographic unit of origin did not. Our results suggest a relatively simple indicator of the likelihood of a translocated individual's survival: heterozygosity.

In a world of rapid environmental change, habitat loss, and species endangerment, translocation of individual plants and animals is becoming increasingly common as a conservation strategy of last resort. For both animals and plants, the long-term success of translocations is often dismal (1–3). There are many possible explanations for this low success, and conservation biologists have proposed several guidelines for improving translocation outcomes, including limiting translocation distances and only exchanging individuals from within the same genetic units to minimize outbreeding depression (4). Although levels of inbreeding and heterozygosity have long been linked to individual survival and fitness (5–7), the relationship between these and translocation success has received little attention. Here, we exploited a long-term dataset for threatened Mojave desert tortoises to generate genomic profiles for 166 tortoises and link those data to individual post-translocation survival.

Mojave desert tortoises (*Gopherus agassizii*) are widely distributed members of the Mojave and Sonoran desert ecological communities west and north of the Colorado River in California, Nevada, Utah, and Arizona, USA (Fig. 1). Although the tortoise is a ubiquitous member of the relatively intact desert ecosystem, decreasing population trends led to the early listing of the species as threatened under the U.S. Endangered Species Act (8). Part of the species' recovery plan includes translocating tortoises salvaged from harmful

anthropogenic activity and habitat destruction to new sites to augment declining populations (9, 10).

Since the establishment of a recovery plan (9), genetic (11, 12) and genomic (4, 13) studies have quantified native population structure within Mojave desert tortoises and have consistently shown that the greatest axis of variation separates the Upper Virgin River and Northeastern Mojave recovery units (hereafter “northern Mojave”) from the rest of the species' distribution (hereafter “southern Mojave”; Fig. 2). Additional fine-scale population structure has been documented within these two regions, leading some researchers to recommend translocations only within these genetically defined populations (4, 11, 14, 15). Others have recommended limiting translocations to specified distances (200 to 276 km) on the basis of spatially distributed genetic structure (16). As is generally the case, these recommendations have assumed that moving animals within, but not between, genetic units (major clades or genetically related metapopulations) should be the guiding principle.

Following the accumulation of hundreds of displaced tortoises at the Desert Tortoise Conservation Center (DTCC) in 1996, the 100-km² Large-Scale Translocation Site (LSTS) was established. The LSTS is located in the Ivanpah Valley near Jean, Nevada, within the natural range of the tortoise, and is surrounded by either a tortoise-barrier fence or relatively inhospitable mountains (Fig. 1 and fig. S1). Because the majority of the tortoises received at the DTCC were captives (many from Nevada's free pet tortoise pickup program), most individuals lacked reliable information on their native site of origin. Between 1997 and 2014, ~9105 tortoises (~50.2% of which were adults) of unknown provenance were translocated to the LSTS, where they intermingled with an estimated 1450 adult local tortoises that were natural residents at the site (17). Most native

and translocated tortoises in the LSTS have since died, consistent with steep declines in neighboring populations and likely furthered by high post-translocation densities and less comprehensive health screening during the first decade of the translocation program. However, roughly 350 adults were estimated by line-distance surveys to be alive in 2015 (18).

By 2016, there were three classes of LSTS tortoises: known-living and known-dead translocated individuals, and unmarked individuals presumed to be pre-translocation residents. For simplicity we refer to these as living, dead, and resident, respectively. Because no information is available on the origins of translocated tortoises, we generated restriction site-associated DNA sequencing (RADseq) genomic data and used these data to infer the geographic origins of a set of living and dead tortoises by comparing them to 270 low-coverage Mojave desert tortoise genomes that were field-collected from across the species' range (13) (Fig. 1).

By comparing living and dead tortoises from the LSTS, we explicitly address three questions central to assisted migration and genetic rescue efforts: (i) Do tortoises from more distant localities have lower survival fitness than those from nearby sites of origin? (ii) Do within-genetic unit (northern or southern Mojave) translocations enjoy greater survival than those that cross this primary genetic boundary? (iii) Are tortoises with higher overall heterozygosity, measured at deeply sequenced RAD loci, more likely to survive than less genetically variable individuals?

Mapped RADseq reads from the LSTS tortoises contained 6,711,580 of the 36,138,619 single-nucleotide polymorphisms (SNPs) found among 270 low-coverage Mojave desert tortoise genomes (13). We empirically evaluated several approaches to infer the place of origin of LSTS tortoises (18). Placing 12 known-origin calibration samples at the location of their genetic nearest neighbors resulted in a mean error of 61.7 km (SD = 60.2) from their true origin. A multi-individual, centroid-based placement approach using the eight closest genetic relatives reduced the mean placement error to 41.7 km (SD = 25.0). Finally, the optimal combined approach resulted when individuals with heterozygosity (π) values of <0.0020 were placed with their closest genomic match (presumably their closest relative) and individuals with π values of >0.0020 were placed at the centroid of their closest eight genetic relatives. This combined approach resulted in a mean error of 35.6 km (SD = 27.7). The combined method is thus more accurate, but that accuracy may result from overfitting a complex model with only 12 calibration animals. Countering this concern, we note that 87% of all LSTS tortoises had π values of <0.0020 and hence were geolocated only on the basis of their genetic nearest neighbors; moreover, given the very

¹Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, USA. ²Department of Life, Earth, and Environmental Sciences, West Texas A&M University, Canyon, TX 79016, USA. ³U.S. Fish and Wildlife Service, Desert Tortoise Recovery Office, Reno, NV 89502, USA. ⁴La Kretz Center for California Conservation Science, Institute of the Environment and Sustainability, University of California, Los Angeles, CA 90095, USA.

*Corresponding author. Email: pete.a.scott@gmail.com

low coverage ($\sim 1.5\times$) of our dataset of 270 reference tortoises, we expected that more heterozygous LSTS tortoises would be difficult to associate with a true closest relative because heterozygosity is underestimated at $\sim 1.5\times$ coverage. Thus, averaging across a set of close matches should outperform a single match for those relatively heterozygous individuals. Because the dual method had the lowest combined error for the 12 calibration samples, we used it to determine the geographic and genetic provenance of all LSTS tortoises.

We calculated probable geographic origins for 166 living and dead LSTS tortoises that were matched for release year and sex (Fig. 2). Sixty-eight of the 79 living tortoises and 78 of the 87 dead tortoises were genomically placed in the geographically proximal northern Mojave genetic unit, and the remainder in the more distant southern Mojave unit (13). We found no difference in the proportion of northern versus southern Mojave desert tortoises that died or survived after translocation [χ^2 (df = 1, $N = 166$) = 1.18, $P = 0.28$]. We also detected no effect of geographic distance between the site of origin and the LSTS for individuals that died or survived after translocation (Fig. 3B; $P = 0.83$).

In contrast, we found that LSTS-translocated survivors had much higher individual heterozygosity when they were compared to those that died (Fig. 3A; mean π of living tortoises = 0.00180, mean π of dead tortoises = 0.00146; $P = 0.00000005$), indicating that individual genetic diversity predicted translocation success after accounting for release year and sex. The mean heterozygosity of the survivors was 23.09% greater than that of a matched set of tortoises that died over the same period. Although the importance of genetic diversity (or its presumed proxy, population size) of stock populations for translocation has been the subject of a few recent studies, this small body of work has yielded contradictory results on the role of population-level variation in translocation success, with some evidence for negligible importance (19–21) and other evidence for substantial importance (22, 23). This discrepancy may stem from the assumption that population-level diversity is an accurate proxy for individual heterozygosity, which has not been tested. Our results demonstrate that individual heterozygosity, rather than population size or overall diversity, is a key, easily measured metric for predicting translocation success.

To explore the possibility that our observed relationship between post-translocation survival and heterozygosity is an artifact of sample age or condition, we confirmed that read depth, sample age, influence of outlier loci, and library complexity are not correlated with heterozygosity (18). Hence, we conclude that heterozygosity itself is a strong indicator of post-translocation

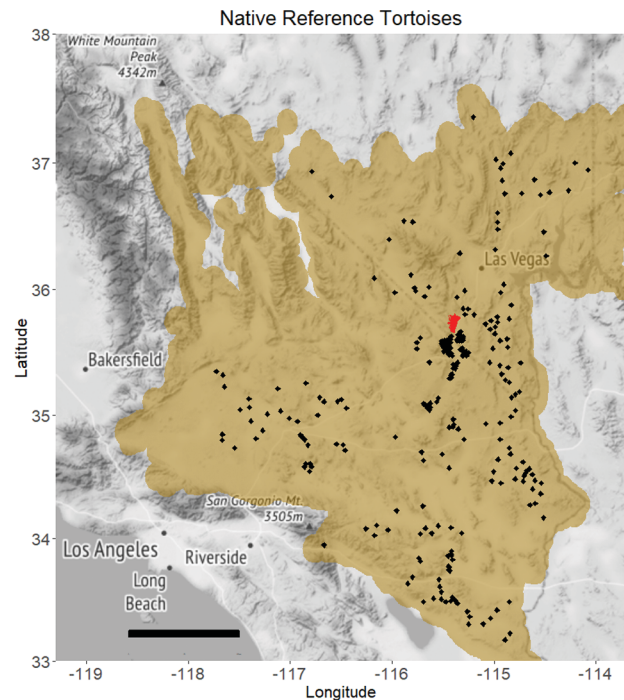


Fig. 1. Map showing the approximate historical distribution of the Mojave desert tortoise (*Gopherus agassizii*). The area in tan represents the distribution; black diamonds denote locations of 270 native low-coverage genome samples. The LSTS is shown as a red polygon. We lack samples for inference only from the northwest portion of the historical distribution for the species. Scale bar, 100 km.

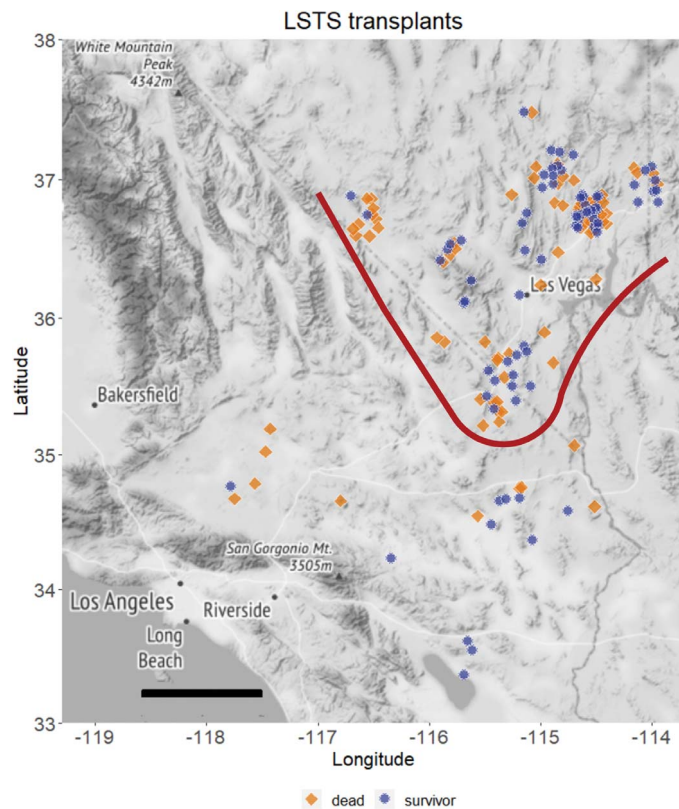


Fig. 2. Map showing the inferred origins of 79 translocation survivors and 87 sex- and release year-matched translocated dead tortoises. Points are slightly jittered for visual clarity. The red line shows the boundary between northern and southern Mojave tortoise genetic units. The inferred points of origin of tortoises that died and survived are shown as orange and blue diamonds, respectively. Scale bar, 100 km.

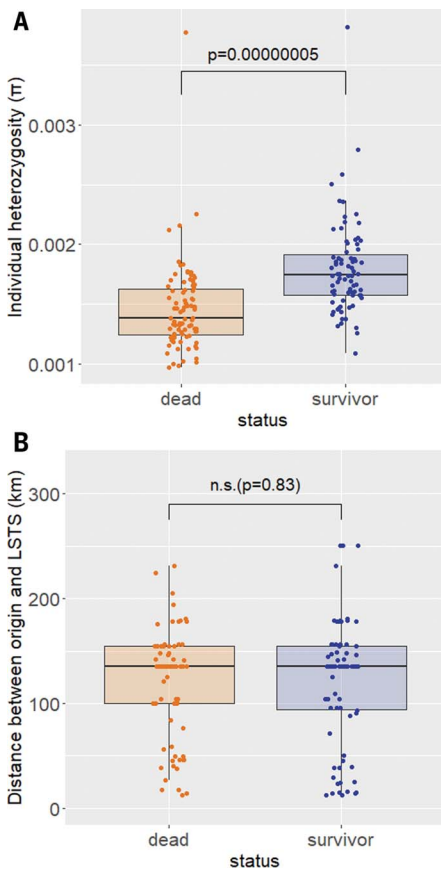


Fig. 3. Box-and-whisker plots showing the mean and distribution of individual heterozygosity. (A and B) Heterozygosity π (A) and straight-line translocation distances (B) of LSTS mortalities and survivors. Reported P values are based on t tests (n.s., not significant).

survival. We are not suggesting that individual heterozygosity should be the only criterion for deciding which individuals to translocate: Local ecology, disease exposure history, and individual condition are some of the other factors that are often critical, and we stress the importance of verifying these results in other systems. We also emphasize that our RAD data, although extensive, are only a proxy for the entire genome, and that additional studies with high-coverage whole-genome resequencing could help to determine whether survivorship is linked to runs of homo- or heterozygosity, level of individual inbreeding, or specific loci under strong selection. Regardless, one of the advantages of individual heterozygosity is that it can now be easily and economically measured, with reduced representation approaches or at the whole-genome level for most organisms, making it a particularly attractive tool for managers and decision-makers.

Our analysis of LSTS survivors and mortalities, combined with detailed landscape genomic data for the entire species, indicates that

matching the geographical provenance of translocated tortoises to their new site had virtually no predictive power in determining survival fitness over a decadal time scale. However, individual heterozygosity did, with more heterozygous individuals outsurviving less heterozygous ones. The overall benefits of genome-level variability have long been suggested as a key fitness component (7, 24), and we were able to use long-term field data to directly show this benefit under natural conditions. Although field observations have confirmed living status for only 3.8% of translocated tortoises, our data indicate that even under these severe conditions, the more variable tortoises outsurvived their less variable conspecifics, which suggests that these genetically variable individuals were better able to survive after translocation. To confirm that individual heterozygosity is responsible for the increase in survival requires understanding the proximate reasons for the massive mortality seen in both translocated and resident tortoises, and we currently lack that knowledge. Long-term monitoring found that the annual mean declines of 7.4% and 9.2% at the individual sites closest to the LSTS occurred between 2004 and 2014 (25), corresponding to population reductions of roughly 57% and 65% over 11 years. These declines, although not fully understood, are at least partially attributed to severe regional drought (26), which has been associated with sharp increases in mortality of Mojave desert tortoises (27–29). Given our current understanding, we can only speculate that drought, combined with high post-release densities, disease, and/or the ecological disruption associated with translocation, may be contributing to the high LSTS mortalities.

However, even without a proximate mechanism, our results suggest that an optimal strategy of assisted migration could be to prioritize moving the most genomically variable individuals, rather than current practice based solely on geographic or genetic similarity. Given the future climate and anthropogenic changes anticipated across the region, assisted migration will likely be a key component of management of desert tortoises and many other declining or endangered species, and our data indicate that targeting the most genomically variable individuals can enhance the success of this work. Future research aimed at understanding the proximate reasons for this increased survival at the genetic and physiological level constitutes an important next step for more efficient conservation-based translocation success.

REFERENCES AND NOTES

- B. B. Beck, L. G. Rappaport, M. S. Price, A. Wilson, in *Creative Conservation: Interactive Management of Wild and Captive Animals*, P. J. S. Olney, G. M. Mace, A. T. C. Feistner, Eds. (Chapman and Hall, 1994), pp. 265–284.
- J. Fischer, D. B. Lindenmayer, *Biol. Conserv.* **96**, 1–11 (2000).

- S. Godefroid *et al.*, *Biol. Conserv.* **144**, 672–682 (2011).
- S. Sánchez-Ramírez *et al.*, *Sci. Rep.* **8**, 11231 (2018).
- D. H. Reed, R. Frankham, *Conserv. Biol.* **17**, 230–237 (2003).
- J. R. Chapman, S. Nakagawa, D. W. Coltman, J. Slate, B. C. Sheldon, *Mol. Ecol.* **18**, 2746–2765 (2009).
- M. Szulkin, N. Bierne, P. David, *Evolution* **64**, 1202–1217 (2010).
- U.S. Fish and Wildlife Service, *Fed. Regist.* **55**, 12178–12191 (1990).
- U.S. Fish and Wildlife Service, *Desert Tortoise (Mojave Population): Recovery Plan* (1994).
- U.S. Fish and Wildlife Service, *Revised Recovery Plan for the Mojave Population of the Desert Tortoise (Gopherus agassizii)* (2011).
- R. W. Murphy, K. H. Berry, T. Edwards, A. M. McLuckie, *Chelonian Conserv. Biol.* **6**, 229–251 (2007).
- B. E. Hagerly, C. R. Tracy, *Conserv. Genet.* **11**, 1795–1807 (2010).
- H. B. Shaffer *et al.*, bioRxiv 195743 [preprint], 29 September 2017.
- T. Edwards, C. R. Schwalbe, D. E. Swann, C. S. Goldberg, *Conserv. Genet.* **5**, 485–499 (2004).
- T. Edwards, K. Berry, *Conserv. Genet.* **14**, 649–659 (2013).
- R. C. Averill-Murray, B. E. Hagerly, *Chelonian Conserv. Biol.* **13**, 35–41 (2014).
- R. Tracy, P. Medica, P. S. Corn, *Translocation Long-Term Monitoring, Tortoise Density Evaluation, and Establishment of New LSTSs* (Clark County, Nevada, Desert Conservation Program, 2006).
- See supplementary materials.
- B. A. Lawrence, T. N. Kaye, *Restor. Ecol.* **19**, 166–176 (2011).
- M. C. Yates, D. J. Fraser, *Evol. Appl.* **7**, 871–882 (2014).
- M. C. Yates, E. Bowles, D. J. Fraser, *Proc. R. Soc. B* **286**, 20191989 (2019).
- G. Bowman, C. Perret, S. Hoehn, D. J. Galeuchet, M. Fischer, *J. Ecol.* **96**, 1056–1064 (2008).
- C. G. Oakley, *Evol. Appl.* **6**, 823–831 (2013).
- B. Hansson, L. Westerberg, *Mol. Ecol.* **11**, 2467–2474 (2008).
- L. J. Allison, A. M. McLuckie, *Herpetol. Conserv. Biol.* **13**, 433–452 (2018).
- U.S. Drought Monitor; www.drought.gov/drought/states/nevada.
- C. C. Peterson, *Biol. Conserv.* **70**, 101–108 (1994).
- K. M. Longshore, J. R. Jaeger, J. M. Sappington, *J. Herpetol.* **37**, 169–177 (2003).
- J. E. Lovich *et al.*, *Biol. Conserv.* **169**, 214–224 (2014).
- J. Sambrook, D. W. Russell, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, 2001).
- N. J. Bayona-Vásquez *et al.*, *PeerJ* **7**, e7724 (2019).
- B. K. Peterson, J. N. Weber, E. H. Kay, H. S. Fisher, H. E. Hoekstra, *PLOS ONE* **7**, e37135 (2012).
- A. M. Bolger, M. Lohse, B. Usadel, *Bioinformatics* **30**, 2114–2120 (2014).
- E. Aronesty, ea-utils: Command-line tools for processing biological sequencing data (2011); https://github.com/ExpressionAnalysis/ea-utils.
- M. Tollis *et al.*, *PLOS ONE* **12**, e0177708 (2017).
- H. Li, arXiv 1303.3997 [q-bio.GN] (16 March 2013).
- H. Li *et al.*, *Bioinformatics* **25**, 2078–2079 (2009).
- T. S. Korneliussen, A. Albrechtsen, R. Nielsen, *BMC Bioinformatics* **15**, 356 (2014).
- D. A. Eaton, *Bioinformatics* **30**, 1844–1849 (2014).
- R Core Team, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2019).
- M. Foll, O. Gaggiotti, *Genetics* **180**, 977–993 (2008).
- M. Foll, M. C. Fischer, G. Heckel, L. Excoffier, *Mol. Ecol.* **19**, 4638–4647 (2010).
- M. C. Fischer, M. Foll, L. Excoffier, G. Heckel, *Mol. Ecol.* **20**, 1450–1462 (2011).
- M. Plummer, N. Best, K. Cowles, K. Vines, *R News* **6**, 7–11 (2006).
- A. Raj, M. Stephens, J. K. Pritchard, *Genetics* **197**, 573–589 (2014).

ACKNOWLEDGMENTS

The Great Basin Institute conducted surveys and collected blood samples from the LSTS. We thank E. McCartney-Melstad, P. Ralph, C. R. Tracy, and members of the Shaffer lab at UCLA for input and advice, and P. Kareiva and D. Simberloff for thoughtful comments on earlier drafts of this manuscript. The findings and conclusions

provided in this article are those of the authors and do not necessarily represent the views of their affiliated organizations. All field work and sample collection was carried out under USFWS recovery permit TE-108507-2 and with written permission from the Bureau of Land Management to L.J.A. **Funding:** Partial funding was provided by the Bureau of Land Management Las Vegas Field Office and the NSF. **Author contributions:** All authors contributed to conceptualizing the main experiment based on sequencing LSTS tortoises. L.J.A. and K.J.F. coordinated collection of LSTS samples and identification of tortoise histories. P.A.S. conducted all laboratory preparation, bioinformatics processing, and analyses.

P.A.S. and H.B.S. wrote the manuscript with input from R.C.A.-M., L.J.A., and K.J.F. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** All sequence data are available in the NCBI Short Read Archive (SRA accession: PRJNA638160). Data on yearly tortoise releases and resightings are available in the supplementary text: "Population density estimates in the LSTS and surrounding regions". These data are accompanied by additional information regarding pre-LSTS population estimates, regional tortoise declines, and our understanding that long-term drought is a causal force in these declines.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/370/6520/1086/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S8
Tables S1 and S2
References (30–45)

4 February 2020; resubmitted 11 September 2020
Accepted 20 October 2020
10.1126/science.abb0421

Individual heterozygosity predicts translocation success in threatened desert tortoises

Peter A. Scott, Linda J. Allison, Kimberleigh J. Field, Roy C. Averill-Murray and H. Bradley Shaffer

Science **370** (6520), 1086-1089.
DOI: 10.1126/science.abb0421

Survival of the most variable

As more species become highly threatened because of human activity, there has been an increasing push to understand how best to reintroduce or translocate individuals from wild or captive populations. Suggestions have varied from choosing individuals from the most environmentally similar regions to choosing those that might have the best ability to adapt to new environments. Scott *et al.* used long-term data collected during translocations of Mojave Desert tortoises, including animals formerly kept as pets, to test these questions. Although the overall rates of survival for all tortoises at the site (both reintroduced and native) were extremely low, translocated individuals with the highest heterozygosity survived at much higher rates than those determined to be similar to the target population.

Science, this issue p. 1086

ARTICLE TOOLS

<http://science.sciencemag.org/content/370/6520/1086>

SUPPLEMENTARY MATERIALS

<http://science.sciencemag.org/content/suppl/2020/11/23/370.6520.1086.DC1>

REFERENCES

This article cites 41 articles, 3 of which you can access for free
<http://science.sciencemag.org/content/370/6520/1086#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 2020, American Association for the Advancement of Science