# **Northwest Science Notes**

The purpose of Notes is to publish papers typically less than five pages long. No specific format or content is required for articles published as Notes, but all will be peer-reviewed and must be scientifically credible. Authors may contact the Editor about the suitability of manuscripts for this section.

Anthony P. Clevenger<sup>1</sup>, Western Transportation Institute, Montana State University, PO Box 174250, Bozeman, Montana

## Mitigating Highways for a Ghost: Data Collection Challenges and Implications for Managing Wolverines and Transportation Corridor

#### Abstract

Research provides transportation agencies with evidence-based data to guide the planning and design of crossing structures that effectively link critical habitats and populations. To date, research has focused on a range of mammal species. However, for rare-occurring, wide-ranging species such as wolverines (*Gulo gulo*), collecting the required information can be challenging. Highway crossing structures have been recommended as a conservation strategy for wolverines in the northern U.S. Rocky Mountains. However, there is virtually no information describing wolverine response to crossing mitigation. I describe 15 years of continuous year-round monitoring (1996-2012) of wolverine response to highway mitigation in Banff National Park, Alberta. Crossing structures were monitored using track pads and cameras. Wolverines were detected using crossing structures 10 times. Nine crossings occurred at wildlife underpasses and one at a wildlife overpass. The first detected passage occurred in 2005. Three crossings were recorded during the same crossing check in 2010 and 2011, suggesting use by the same individual of the structures. Few conclusions can be drawn regarding the attributes of crossing structures that facilitate passage of wolverines. Given the scarcity of crossing structures within wolverine range, it will be difficult to collect sufficient information in the short term for this rare and elusive species. Given the proposal to list wolverines under the Endangered Species Act, transportation departments and land managers should begin proactively identifying critical habitat linkages across highways in wolverine range and opportunities for highway mitigation in the short and long term.

Keywords: Banff National Park, Gulo gulo, roads, wildlife crossing structures, wolverine

#### Introduction

Ecological connectivity at a landscape scale is becoming increasingly important in the face of a changing climate (Heller and Zavaleta 2009). Local-scale corridors such as wildlife crossing structures may play an important role in allowing animals to adapt and respond to a warming climate. Research that identifies important attributes of wildlife crossing structures (underpasses and overpasses) that facilitate connectivity and dispersal for key fragmentation-sensitive species is needed to ensure local-scale habitat linkages will be able to mitigate continental-scale bottlenecks (Crooks and Sanjayan 2006, Clevenger 2012).

Monitoring and research provides transportation agencies with evidence-based data to guide the planning and design of crossing structures that effectively link critical habitats and populations (Clevenger and Huijser 2011, Gagnon et al. 2011, Van Manen et al. 2012). To date, research has focused on a range of migratory and nonmigratory mammals in different landscapes and biomes including Florida panthers (*Felis concolor* 

Author to whom correspondence should be addressed. Email: apclevenger@gmail.com

*coryi*, Jansen et al. 2010), bobcats (*Lynx rufus*, Cain et al. 2003), cougars (*F. concolor*, Beier 1995), moose (*Alces alces*, Olsson and Widen 2008), bears (*Ursus* sp.; Clevenger and Wierzchowski 2006, Lewis et al. 2011, Van Manen et al. 2012) elk (*Cervus elaphus*, Gagnon et al. 2011) and others (Van Wieren and Worm 2001, Bond and Jones 2008). However, for rare-occurring, wide-ranging species, collecting the required information with sufficient sample sizes to draw inference-based conclusions can be exceptionally difficult and time-consuming.

Wolverines (Gulo gulo) naturally occur in low numbers and have some of the lowest rates of reproduction of any terrestrial mammal species (Banci and Harestad 1990, Inman et al. 2012). Because of their low-densities and association with rugged and remote habitats (Copeland et al. 2007, Krebs et al. 2007), obtaining information on wolverine occurrence and ecological requisites has proved challenging (Ruggiero et al. 2007). Wolverines are becoming recognized as genuine indicators of healthy, connected ecosystems due to their sensitivity to human disturbance and needs for large areas and intact habitats. (Schwartz et al. 2009, Copeland et al. 2010). Further, in February 2013, the U.S. Fish and Wildlife Service proposed to list wolverines in the contiguous United States as threatened (U.S. Fish and Wildlife Service 2013).

Highway expansion projects are occurring at a rapid pace within wolverine range in the northern U.S. Rocky Mountains. Similarly, road and highway upgrades are planned for energy development activities within their range in Alberta and British Columbia (Province of British Columbia 2011, Government of Alberta 2012). Highway crossing structures have been identified as one of three recommended conservation strategies for the future conservation of the wolverine metapopulation in the northern U.S. Rocky Mountains (Inman 2013). I am not aware of published literature (peer-reviewed or grey) anywhere in the world describing wolverine response to highway mitigation measures, such as wildlife crossing structures and fencing.

There are at least two reasons for the dearth of information. First, although wildlife crossing

structures are becoming more common on North American highways, not all are systematically monitored for wildlife use. Monitoring is rarely conducted for more than 1-2 years; thus, sufficient time scales for wolverine use to occur haven been inadequate. Second, within wolverine range there are very few highways with wildlife crossing structures, and only one is located in core wolverine habitat as defined by persistent snow pack modeling (Copeland et al. 2010, McKelvey et al. 2011). Given the species low density and tendency to avoid transportation infrastructure (Austin 1998, Copeland 1996, Packila et al. 2007), collecting information on wolverine use of crossing structures now or in the future will be challenging at best and inevitably result in extremely small samples sizes. The Trans-Canada Highway (TCH) in Banff National Park (BNP), Alberta is one of the few places in the world today where wolverines may be detected using wildlife crossing structures.

Understanding how highways affect wolverine dispersal and means of successfully mitigating road impacts will be a critical part of local and continental scale conservation strategies (Clevenger 2012). Information on wolverine use of crossing structures, albeit sparse, will be of value to transportation and natural resource agency decision makers responsible for mitigating transportation projects within wolverine range today and in the future. My purpose of this paper is to describe long-term, year-round monitoring of wolverine response to highway mitigation measures (crossing structures and fencing) in a critical fracture zone where maintaining highway permeability is a conservation concern (Weaver et al. 1996).

## **Study Area**

The TCH in the Canadian Rocky Mountains has long been recognized as a lethal barrier to wildlife and a potential bottleneck for population connectivity at local and trans-boundary scales (Weaver et al. 1996, Proctor et al. 2012). Over 30 years ago, safety and logistical considerations compelled planners to upgrade the TCH within BNP from two to four lanes (i.e., twinning), beginning from the eastern boundary of the park and working west (Clevenger and Waltho 2005). In each phase, large mammals were excluded from the road with a 2.4-m-high fence erected on both sides of the highway. Underpasses were also built to allow wildlife to cross the road. The first 27 km of highway twinning included 11 wildlife underpasses and was completed by 1988 (Figure 1). The next 18 km section was completed in late 1997 with 10 additional wildlife underpasses and 2 wildlife overpasses (Ford et al. 2010). The final 38 km of twinning to the western park boundary at the



Figure 1. Wildlife crossing structures and their respective highway twinning phases along the Trans-Canada Highway in Banff National Park, Alberta.

Continental Divide and British Columbia-Alberta border will be completed in 2013 and consist of 21 additional wildlife crossing structures, including four 60-m wide wildlife overpasses.

BNP is unrivalled in terms of the number and diversity of wildlife crossing structures and associated biological data on wildlife distribution, movement and ecology. Eleven species of large mammals have been detected using the crossing structures more than 130,000 times during more than 15 years of systematic year-round monitoring (Clevenger et al. 2012). Mitigation efforts during the last 25 years have helped restore habitat connectivity across large sections of this major transportation corridor. The measures have been effective at reducing highway-related mortality of large mammals (Clevenger et al. 2001), contributing to dispersal and gene flow among grizzly (U. arctos) and black bears (U. americanus; Sawaya et al. [in press]) and provided evidence-based guidelines for future crossing structure designs in BNP and elsewhere (Clevenger and Waltho 2005; Clevenger and Huijser 2011).

#### Methods

Systematic year-round monitoring of the BNP crossing structures began in November 1996 (Clevenger and Waltho 2005). Monitoring consisted of checking the crossing structures and recording animal movement across raked track pads. Track pads spanned the width of the wildlife underpasses, were generally  $\approx 2$  m wide, and were set perpendicular to the direction of animal movement. At wildlife overpasses a single, 4-m-wide

track pad was set across the center and motionsensitive cameras were used to supplement track pad data. Tracking material consisted of a dry, loamy mixture of sand, silt and clay, 1-4 cm deep. Each crossing structure was visited every two to four days throughout the year. Observers identified tracks to species, estimated the number of individuals, their direction of travel (northbound or southbound across the TCH) and whether they moved through the crossing structure. Since 2005, motion-sensitive cameras were increasingly used to supplement track pads to monitor species use of the crossing structures (Ford et al. 2009). These cameras (Reconyx Inc., Holmen, Wisconsin) also provide information on time, animal behavior, and ambient temperature during each crossing event. Information on wolverine road-related mortalities and fence intrusions within the study area were collected opportunistically during the study period and with the help of the Parks Canada staff.

#### Results

Wolverines have been detected using the BNP crossing structures 10 times in the last 15 years. Nine of the recorded crossings occurred at wild-life underpasses, while one occurred at a wildlife overpass (Table 1). The first recorded passages (n=3) were detected during one monitoring check on 23 December 2005 at the Redearth Creek underpass (northbound and southbound) and Copper underpass (northbound). Three years later the fourth crossing was detected on 25 April 2008 at the Wolverine Creek underpass (southbound). In 2010, three wolverine crossings were

 TABLE 1. Attributes of crossing structures where wolverines have been detected crossing the Trans-Canada Highway, Banff National Park, Alberta. All structures span four lanes of highway.

Crossing structure	Location- Km #	Design	Dimensions (width x height)	Number of detections
Wolverine overpass	27	Overpass	50-m wide	1
Wolverine Creek	29	Creek bridge underpass	11 m x 2.5 m	2
Pilot	34	Box culvert underpass	3.0 m x 2.4 m	1
Redearth Creek	37	Creek bridge underpass	11.4 m x 2.2 m	2
Copper	39	Elliptical culvert underpass	7.0 m x 4.0 m	1
Johnston	42	Box culvert underpass	2.4 m x 3.0 m	1
Castle	44	Elliptical culvert underpass	7.0 m x 4.0 m	1
Moraine Creek	64	Creek bridge underpass	16.0 m x 2.0 m	1

observed during one monitoring check (12 April) at Wolverine Creek underpass (southbound), Pilot underpass (northbound) and Johnston underpass (southbound; Figure 1). Three crossings were detected in 2011: 16 February at Castle underpass (northbound), 25 February at Moraine Creek underpass (southbound) and 16 November at Wolverine Overpass (northbound).

Parks Canada records of wildlife mortalities indicate that since 1980, five wolverines have died on highways in Banff and Kootenay National Parks; two adult males on the unmitigated TCH (1988, 1989), a subadult female near Marble Canyon (2012) and one of unknown gender and age on Highway 93 South near Vermilion Pass (1981). There have been four known incidents where wolverines have been documented climbing the TCH fence to cross or access the TCH right-of-way (three in 2011, one in 2012). All of the intrusions have occurred within 700 m of the nearest crossing structure.

#### Discussion

Of the 10 detected passages so far, we are unable to identify the number of individuals and gender of wolverines using the TCH crossing structures. The two monitoring checks (23 December 2005, 12 April 2010) where three passages were detected at the crossing structures suggest these were likely made by the same individual moving back and forth across the TCH. The tracks appeared to be the same age and moved in alternating directions through the structures.

The majority of 15+ years of monitoring occurred on the first 45 km of TCH (Phase 1, 2, 3A, Figure 1). Unlike the final 38-km section located near the Continental Divide (Phase 3B, Figure 1), this section lies in low-elevation montane habitat not previously considered optimal wolverine habitat according to the BNP ecological land classification map (Holryod and Van Tighem 1983). This map was created in the 1970s and was based on anecdotal data and what little was known about wolverine habitat at that time. Wolverines have been detected on both sides of the TCH, and relatively close to it, as part of a park-wide noninvasive wolverine survey from 2010-2013 (A. Clevenger, unpublished data). These survey and crossing data suggest that wolverines avoid or seldom use wildlife crossing structures (possibly at random). Low use up until now may be attributed to avoidance of the TCH corridor or potential interactions with wolf packs occupying the Bow Valley (Inman et al. 2012). Future monitoring of the Phase 3B crossing structures will provide needed clarity on whether wolverines will use crossing structures and the effects of habitat and interspecific interactions.

Despite the small sample size we present after 15 years of Banff research, we are acutely aware of their limitations but also their uniqueness and value for practitioners and decision makers charged with highway mitigation schemes in wolverine range. The TCH has been mitigated in successive phases from 1982 to present. During that time there have been no wolverine mortalities on the mitigated highway, although two reported mortalities occurred in 1988 and 1997 while it was unfenced and unmitigated. Fencing does not preclude animals from occasionally climbing the highway fence (Clevenger et al. 2001). There have been four known incidents where wolverines were documented climbing the TCH fence to cross or access the TCH right-ofway. The relatively low incidence of mortality and fence-climbing may in part be due to their aversion to roads and highway corridors. Ongoing research in Banff, Yoho, and Kootenay National Parks will help to better understand the population-level effects of the TCH on wolverine dispersal, population genetics, and landscape connectivity (A. Clevenger, unpublished data).

As the construction of TCH crossing structures proceeds further west and ultimately into Yoho National Park, British Columbia, it enters subalpine habitats and will become the first attempt ever in North America to introduce highway mitigation at the Continental Divide, in core wolverine habitat according to snow persistence models (Copeland et al. 2010). This high-elevation ecosystem is doubly important given it is acutely affected by a warming climate and its north-south axis is bisected by east-west transportation corridors here and in other parts of the Rocky Mountain cordillera in Canada and the United States (Graumlich and Francis 2010). The need for this information is particularly critical given current highway expansion plans in wolverine habitat in the northern U.S. Rocky Mountains.

From the relatively sparse data we have collected over 15 years, we are unable to draw any sound conclusions or even analyze generally the attributes that facilitate passage of wolverines, as we have done for other large mammals previously (Clevenger and Waltho 2005). We may never have sufficient data from BNP alone to conduct a robust analysis, and it is unlikely that other study areas will be available in the near future. The difficulty lies in finding highway study sites within wolverine range having crossing structures and being monitored over sufficient time periods to collect robust sample sizes. Given the difficulties of securing data on wolverine response to crossing structures, a meta-analysis using data collected from multiple highway monitoring sites within wolverine range will likely best provide information to identify crossing structure needs for this rare and elusive species.

The current lack of information and urgency for information is of increasing concern, given the 2013 proposed listing under the Endangered Species Act (ESA; U.S. Fish and Wildlife Service 2013) and the possible reintroduction to Colorado (R. Inman, Wildlife Conservation Society, personal communication). Should wolverines become listed under the ESA and/or reintroduced into Colorado, transportation departments will be required to mitigate their projects for wolverine

## Literature Cited

- Austin, M. 1998. Wolverine winter travel routes and response to transportation corridors in Kicking Horse Pass between Yoho and Banff National Parks. M.S. Thesis. University of Calgary, Calgary, Alberta.
- Banci, V., and A. Harestad. 1990. Home range and habitat use of wolverines in Yukon, Canada. Holarctic Ecology 13:195-200.
- Beier, P. 1995. Dispersal of juvenile cougars in fragmented habitat. Journal of Wildlife Management 59:228-237.
- Bond, A. R., and D. N. Jones. 2008. Temporal trends in use of fauna-friendly underpasses and overpasses. Wildlife Research 35:103-112.
- Cain, A. T., V. R. Tuovila, D. G. Hewitt, and M. E. Tewes. 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. Biological Conservation 114:189-197.

movement. Maintaining wolverine populations in the largest remaining areas of contiguous habitat in the southern portion of their range and facilitating connectivity among habitat patches is a recommended conservation measure to help sustain a viable wolverine population in the U.S. (McKelvey et al. 2011). Transportation departments and land managers should begin proactively identifying critical dispersal corridors across highways in the remaining areas of contiguous habitat, adjacent land securement issues and any potential opportunities for highway mitigation in the short and long term.

### Acknowledgements

Thanks to Trevor Kinley, Omar McDadi and two anonymous reviewers for edits to an early draft. Ben Dorsey created the study area figure and provided geographic information system (GIS) assistance. The long-term project has been supported by Parks Canada, Western Transportation Institute at Montana State University, Woodcock Foundation, Henry P. Kendall Foundation, and Wilburforce Foundation. Stephen Woodley championed our research within Parks Canada. I thank the many field assistants and volunteers for their help checking crossing structures, raking track pads year-round, and helping with the time-consuming task of classifying photographs from the crossing structures.

- Clevenger, A. P. 2012. Mitigating continental scale bottlenecks: How small-scale highway mitigation has large-scale impacts. Ecological Restoration 30:300-307.
- Clevenger, A. P., and N. Waltho. 2005. Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. Biological Conservation 121:453-464.
- Clevenger, A. P., and J. Wierzchowski. 2006. Maintaining and restoring connectivity in landscapes fragmented by roads. *In* K. Crooks and M. Sanjayan (editors). Connectivity Conservation. Cambridge University Press, New York, NY. Pp. 502-535.
- Clevenger, A. P., and M. P. Huijser. 2011. Wildlife Crossing Structure Handbook, Design and Evaluation in North America, Publication No. FHWA-CFL/ TD-11-003. Department of Transportation, Federal Highway Administration, Washington D.C., USA.

302 Clevenger

- Clevenger, A. P., B. Chruszcz, and K. Gunson. 2001. Highway mitigation fencing reduces wildlife-vehicle collisions. Wildlife Society Bulletin 29:646-653.
- Clevenger A. P., R. Ament, D. Duke and R. Haddock. 2012. Trans-Canada Highway wildlife monitoring and research. Annual report, Year 3 – 2011-12. Unpublished Report on file at Parks Canada Agency, Radium Hot Springs, B.C.
- Copeland, J. 1996. Biology of the wolverine in central Idaho. M.S. Thesis, University of Idaho, Moscow, Idaho.
- Copeland, J., J. Peek, C. Groves, W. Melquist, K. McKelvey, G. McDaniel, C. Long, and C. Harris. 2007. Seasonal habitat assocations of the wolverine in central Idaho. Journal of Wildlife Management 71:2201-2212.
- Copeland, J., K. S. McKelvey, K. B. Aubry, A. Landa, J. Persson, R. M. Inman, J. Krebs, E. Lofroth, H. Golden, J. R. Squires, A. Magoun, M. K. Schwartz, J. Wilmot, C. L. Copeland, R. E. Yates, and R. May. 2010. The bioclimatic envelope of the wolverine (*Gulo gulo*): do climatic constraints limit its geographic distribution? Canadian Journal of Zoology 88:233-246.
- Crooks, K. R. and M. Sanjayan. 2006. Connectivity Conservation. Cambridge University Press, New York, NY.
- Ford, A. T., A. P. Clevenger and A. Bennett. 2009. Comparison of non-invasive methods for monitoring wildlife crossing structures on highways. Journal of Wildlife Management 73:1213-1222.
- Ford, A. T., A. P. Clevenger, K. Rettie. 2010. Banff Wildlife Crossings, Trans-Canada Highway, Alberta— An international public-private partnership. *In* J. P. Beckmann, A. P. Clevenger, M. P. Huijser, and J. A. Hilty (editors). Safe Passages: Highways, Wildlife and Habitat Connectivity. Island Press, Washington DC. Pp. 157-172
- Gagnon, J. W., N. L. Dodd, K. S. Ogren, R. E. Schweinsburg. 2011. Factors associated with use of wildlife underpasses and importance of long-term monitoring. Journal of Wildlife Management 75:1477-1487.
- Government of Alberta. 2012. Inventory of major Alberta projects. Alberta Treasury Board and Enterprise, Government of Alberta, Edmonton, Alberta.
- Graumlich, L., and W. L. Francis, (Eds.). 2010. Moving Toward Climate Change Adaptation: The Promise of the Yellowstone to Yukon Conservation Initiative for addressing the Region's Vulnerabilities. Yellowstone to Yukon Conservation Initiative. Canmore, Alberta.
- Heller, N. E., and E. S. Zavaleta. 2009. Biodiversity management in the face of climate change: A review of 22 years of recommendations. Biological Conservation 142:14-32.
- Holroyd, G. L. and K. J. Van Tighem. 1983. Ecological (biophysical) land classification of Banff and Jasper national parks. Volume 3. The wildlife

inventory. Canadian Wildlife Service, Edmonton, Alberta, Canada. Alberta Institute of Pedology, Publ. M-83-2.

- Inman, R.M. 2013. Wolverine ecology and conservation in the Western United States. Ph.D. Dissertation, Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Inman, R. M., M. L. Packila, K. H. Inman, A. McCue, G. White J. Persson, B. Aber, M.
- Orme, K. Alt, S. Cain, J. Frederick, B. Oakleaf, S. Sartorius. 2012. Spatial ecology of wolverines at the southern periphery of distribution. Journal of Wildlife Management 76:778-792.
- Jansen, D., K. Sherwood, and E. Fleming. 2010. The I-75 project: lessons from the Florida Panther. In J. P. Beckmann, A. P. Clevenger, M. P. Huijser, and J. A. Hilty (editors). Safe Passages: Highways, Wildlife and Habitat Connectivity. Island Press, Washington DC. Pp. 205-221
- Krebs, J., E. C. Lofroth, and I. Parfitt. 2007. Multiscale habitat use by wolverines in British Columbia, Canada. Journal of Wildlife Management 71:2180-2192.
- Lewis, J., J. Rachlow, J. Horne, E. Garton, W. Wakkinen, J. Hayden, and P. Zager. 2011. Identifying habitat characteristics to predict highway crossing areas for black bears within a human-modified landscape. Landscape and Urban Planning 101:99-107.
- McKelvey, K. S., J. P. Copeland, M. K. Schwartz, J. S. Littell, K. B. Aubry, J. R. Squires, S. A. Parks, M. M. Elsner, and G. S. Mauger. 2011. Climate change predicted to shift wolverine distributions, connectivity, and dispersal corridors. Ecological Applications 21:2882-2897.
- Olsson, M. P. O., and P. Widen. 2008. Effects of highway fencing and wildlife crossings on moose *Alces alces* movements and space use in southwestern Sweden. Wildlife Biology 14:111-117.
- Packila, M., R. Inman, K. Inman, and A. McCue. 2007. Wolverine road crossings in western Greater Yellowstone. Unpublished Report on file at Wildlife Conservation Society, North American Program, Bozeman, MT.
- Proctor, M., D. Paetkau, B. McLellan, G. Stenhouse, K. Kendall, R. Mace, W. Kasworm, C. Servheen, C. Lausen, M. Gibeau, W. Wakkinen, M. Haroldson, G. Mowat, C. Apps, L. Ciarniello, R. Barclay, M. Boyce, C. Schwartz, and C. Strobeck. 2012. Population fragmentation and inter-ecosystem movements of grizzly bears in western Canada and the northern United States. Wildlife Monograph 180:1-46.
- Province of British Columbia. 2011. Major projects inventory. Ministry of Jobs, Tourism and Innovation, Victoria, British Columbia.
- Ruggiero, L., K. McKelvey, K. Aubry, J. Copeland, D. Pletscher, and M. Hornocker. 2007. Wolverine

conservation and management. Journal of Wildlife Management 71:2145-2146.

- Sawaya, M., A. P. Clevenger, and S. Kalinowski. *In press*. Wildlife crossing structures connect Ursid populations in Banff National Park. Conservation Biology.
- Schwartz, M., J. Copeland, and N. Anderson. 2009. Wolverine gene flow across a narrow climatic niche. Ecology 90:3222-3232.
- U.S. Fish and Wildlife Service. 2013. Endangered and threatened wildlife and plants: Threatened status for the Distinct Population Segment of the North American wolverine occurring in the contiguous

Received 18 May 2012 Accepted for publication 24 May 2013 United States. Federal Register Vol. 78, No. 23, pp. 7864-7890, February 4, 2013.

- Van Manen, F., M. McCollister, J. Nicholson, L. Thompson, J. Kindal, and M. Jones. 2012. Short-term impacts of a 4-lane highway on American black bears in Eastern North Carolina. Wildlife Monograph 181:1-35.
- Van Wieren, S. E., and P. B. Worm. 2001. The use of a motorway wildlife overpass by large mammals. Netherlands Journal of Zoology 51:97-105.
- Weaver, J. L., P. Paquet, and L. Ruggiero. 1996. Resilience and conservation of large carnivores in the Rocky Mountains. Conservation Biology 10:964-976.