

each has an exposed, single-stranded 'toehold sequence' adjacent to the mRNA-binding sequence, which facilitates binding of the trigger RNA to the riboregulator. This design strategy enables riboregulators to be programmed at will.

The authors demonstrated the potential of toehold riboregulators by independently controlling 12 toehold switches inside one cell. They experimentally and computationally characterized their first-generation library of 168 switches to identify specific parameters that are crucial for the proper performance of switches. These parameters enabled the computer-aided design of toehold switches with predictable performance, which was validated for 13 second-generation switches.

These second-generation devices modulated translation extremely efficiently — protein production was up to 650-fold higher when the switch was on than when it was off. This performance is unmatched for other RNA-based switches, and is typically reached only by devices that exert control at the transcriptional level. Demonstrating the versatility of their devices, Green *et al.* produced switches that detect and report on the presence of endogenous RNA sequences, and programmed cellular behaviour using synthetic trigger RNAs.

Might the potential flexibility of toehold switches be exploited in diagnostics? A follow-up report⁸ examined the diagnostic capability of a toehold switch in which the trigger was Ebola virus RNA, and the mRNA under control encoded an enzymatic 'reporter' protein. The switch was freeze dried in cell-free extracts and stored on paper discs. These paper-based switches could reliably detect the Ebola virus RNA with great sensitivity. Furthermore, the switches worked even after long-term storage at ambient temperature. Although designed for use in bacteria, paper-based toehold switches also worked in combination with mammalian cell extracts as protein-based biosensors that quantified blood glucose levels. In the future, paper-based diagnostics might also be used to detect when RNA molecules such as microRNAs are expressed in patterns that are hallmarks of cancer or metabolic disorders⁹.

Although diagnosis is fundamental to any preventive care strategy, therapeutics are also vital, and future treatment strategies could combine the two. Synthetic gene networks that operate inside designer cell implants can monitor, process and score molecular indicators of disease, and can also coordinate the production of protein-based therapies within the engineered cell. Designer networks have been used for the treatment of gouty arthritis³, obesity⁴ and diabetes⁵ in animal models. So far, therapeutic gene networks have used natural sensor components that might be compatible with the human physiological range, but the design of tailor-made biosensors for

specific molecular indicators of disease remains challenging. Toehold switches may be a good starting point to design biosensors specific for any disease-relevant compound — first for microRNAs⁹, and eventually for mutated mRNA sequences. The integration of synthetically engineered biosensors into synthetic gene networks that diagnose and treat disease could dramatically shape cell-based treatment strategies in this century. ■

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MALARIA

How vector mosquitoes beat the heat

Intensive longitudinal sampling of malaria mosquitoes in the African semi-desert reveals that three morphologically indistinguishable species have distinctive strategies for surviving the dry season. SEE LETTER P.387

NORA J. BESANSKY

The scale-up of interventions against malaria in the past decade has reduced the global death rate of this disease by an impressive 42%. However, more than 600,000 malaria-related deaths still occur each year¹ — 90% of them in sub-Saharan Africa — meaning that malaria remains one of the most significant sources of infectious-disease mortality. Africa has long been recognized as a crucible for malaria-control efforts, owing to its particular blend of widespread and dominant mosquito species that transmit malaria. One of the great mysteries of malariology has been how these vector populations survive the dry season, when there is little water in which the mosquitoes can lay their eggs. In this issue, Dao *et al.*² (page 387) report that they have solved this mystery, but the answer is surprisingly complex, like the vectors themselves.

Three closely related sibling mosquito species belonging to the *Anopheles gambiae* complex are among the most efficient vectors of malaria³ (there are at least seven species in the complex, collectively referred to as *A. gambiae sensu lato* (*s.l.*)). This status is owed to their strong association with humans and their success at exploiting a variety of ecological conditions across tropical Africa, from humid rainforests to the fringes of the Sahara Desert, as long as humans are nearby. However, there is an Achilles heel in the relay of malaria parasites between these vectors and humans — all mosquitoes have an obligate

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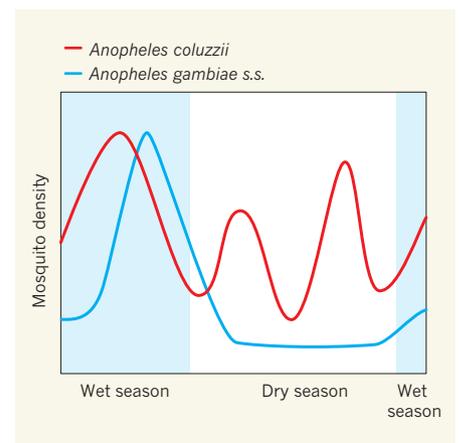


Figure 1 | Species-specific population dynamics. Dao *et al.*² find that average population densities of *Anopheles coluzzii* and *Anopheles gambiae sensu stricto* (*s.s.*) mosquitoes fluctuate seasonally in predictable but distinct patterns. In the wet season, when mosquito breeding sites are abundant and the climate is favourable, densities of both species are high, although *A. coluzzii* achieves its peak population density substantially earlier than *A. gambiae s.s.* does. In the dry season, the *A. gambiae s.s.* population disappears and is not found again until the next wet season, with a slow increase in population density that lags behind that of *A. coluzzii*. By contrast, the *A. coluzzii* population remains in the area during the dry season, but cannot be sampled while the insects are hidden in unknown shelters, leading to apparent troughs. Their emergence from those shelters for two short periods during the dry season is reflected by two peaks in the data.

aquatic immature stage, and in the absence of water, they cannot breed. During the long dry season of the African savannas and the Sahel region, the rains cease for months, surface water evaporates, humidity plummets and temperatures soar. As long as there is no permanent surface water from reservoirs or rivers nearby, malaria transmission becomes undetectable and the local vector mosquitoes also disappear, only to return again with the rains.

Understanding malaria-vector ecology during the dry season, when populations have reached their lowest point, has great strategic significance because deploying mosquito control specifically at those times and places can have the greatest impact. There are two main possibilities for what happens to the mosquitoes during the dry season: long-distance migration to and from refugia where water persists; or stasis, in which the vectors enter a state of dormancy (referred to as aestivation or summer diapause³) that allows them to safely ride out the dry season *in situ*, hidden deep inside (unknown) shelters. Yet finding the disappeared mosquitoes is even harder than it sounds.

In fact, Dao *et al.* did not solve the mystery directly, by physically locating mosquitoes in hiding places or capturing them in the act of long-distance migration, although such efforts are under way³. Instead, their detective work was indirect, using detailed analyses of mosquito population dynamics over time. Although researchers have adopted conceptually similar approaches in the past, the insights that emerge from Dao and colleagues' data were made possible by a sampling effort that is unprecedented both in its detail, allowing the detection of short-lived phenomena, and in its duration, allowing true seasonal patterns to be distinguished from one-off events.

Based in the Sahelian village of Thierola in Mali, the researchers collected mosquitoes from around 120 houses for 2 weeks of every month for 5 years, yielding about 40,000 *A. gambiae s.l.* samples. From time-series analysis of the combined data from all three species, the authors inferred a statistically significant repeating seasonal pattern that was unexpectedly complex. They observed the predicted wet-season peak and mid-dry-season trough in vector density, but this was followed by a surprising rise in density in the late dry season, before another low as the dry season ended.

To make biological sense of these data, Dao *et al.* recognized the importance of splitting *A. gambiae s.l.* into the three genetically defined units found simultaneously in Thierola: *A. gambiae sensu stricto* (*s.s.*), *Anopheles coluzzii* and *Anopheles arabiensis*. Mosquitoes from the three groups are very closely related and cannot be physically distinguished at any stage in their development. All three hybridize occasionally in nature, but the first two — only recently named as species⁶ and

not universally recognized as such — diverged evolutionarily much more recently than other species in the complex.

Despite the relative youth and morphological homogeneity of this species complex, the fact that the species radiations were accompanied by, if not promoted by, differential adaptations to environmental heterogeneities⁷ makes it unlikely that its members would respond uniformly to a common physiological stress. Notwithstanding this expectation, it is striking that, when Dao and colleagues partitioned the data by species, the two closest relatives (*A. coluzzii* and *A. gambiae s.s.*) showed the most distinct population dynamics (Fig. 1). The authors also found that the population density of *A. gambiae s.s.* follows a relatively simple pattern of peak abundance in the wet season and a trough throughout the dry season. By contrast, although the density of *A. coluzzii* also peaks in the wet season, the onset of population growth precedes that of *A. gambiae s.s.* by two months and, far from disappearing in the dry season, two peaks in population density are consistently observed, despite the absence of rain.

Dao *et al.* make the case that these data best fit a model in which *A. coluzzii* persists locally in a form of diapause and emerges from hiding for two short periods. The cues that provoke this emergence are unknown, but could include abiotic factors, such as increases in humidity or temperature, and biotic factors, such as the need to replenish nutritional reserves — for example, by blood feeding without egg maturation, known as gonotrophic dissociation⁴. By contrast, it seems that *A. gambiae s.s.* disappears and, when the rains resume, more slowly recolonizes the area from refugia hundreds of kilometres distant.

Although the population dynamics of *A. arabiensis* were not statistically different from those of *A. gambiae s.s.*, small numbers of *A. arabiensis* were collected each dry season, suggesting that at least a fraction of the population remains in place. Whether this implies that the species uses a mixed strategy of diapause and long-distance migration, as the authors propose, or whether there is some other explanation (such as a different type or greater depth of diapause) will require further investigation.

Final proof for these hypotheses will have to come from catching the mosquitoes in the act. Nevertheless, there is now strong evidence that *A. coluzzii* overcomes the stress of the dry season through local diapause, a strategy that ensures its rapid population expansion at the earliest stages of the rainy season and thereby amplifies disease transmission. The long-distance migration proposed for *A. gambiae s.s.* will also influence the dynamics of disease transmission and vector control, because both processes determine the ability of vector populations to expand their range and invade distant regions. Unfortunately, we know



50 Years Ago

Dr. H. J. Kingsley and Dr. J. E. A. David of Bulawayo have described ... the case of a girl aged 22 months ... She appeared to be completely insensitive to pain ... She was admitted to hospital for investigation and was noticed to have periods of blankness which were thought to be some type of petit mal. Many investigations were made and all results were normal. While she was in hospital her sensitivity to pain was tested and it was found that the child was insensitive to pain almost all over the trunk, limbs and face, and a sterile hypodermic needle could be stuck through the skin to the subcutaneous tissues without any flinching ... Confusion exists in the literature about congenital absence of pain ... Dr. Walter B. Shelley of Philadelphia thinks that these cases are not as rare as is supposed and that there are people who experience coronary thrombosis or a perforating appendicitis, or have babies, without pain. Apparently, where pain is absent, itching is also absent.

From *Nature* 19 December 1964

100 Years Ago

Physics of the Household. By Prof. C. J. Lynde — The author of this book is professor of physics in the Macdonald College, an affiliated college of the McGill University, Montreal, where a school of household science is one of the branches of the institution, and it is for students of household science that the book is written. It presents the subject of physics in close relation to its domestic applications, and abounds in illustrations and examples of household appliances and processes. It should be of great use to science teachers, especially those who have to teach girls.

From *Nature* 17 December 1914

almost nothing about the environmental cues that prompt these processes, the mechanisms responsible for them or even how generalizable these findings are to mosquito populations elsewhere in tropical Africa. Dao and colleagues' work highlights the urgent need for more field studies to answer these fundamental questions. ■

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CONSERVATION

Mind the gaps

New analysis reveals the conservation gains that could be achieved by expanding the global network of protected areas — but also how this may be undermined by land-use change and a lack of international coordination. [SEE LETTER P.383](#)

THOMAS M. BROOKS

Humanity's best tool for safeguarding nature is the establishment of protected areas¹. Such areas currently cover more than 15% of the terrestrial realm and thus are one of the most extensive uses of the world's land². The Aichi Biodiversity Target 11 — set in 2010 under the Convention on Biological Diversity, a multilateral treaty with 194 parties — states that this proportion should be increased to 17% by 2020. Progress towards Target 11 was a key focus when the international protected-areas community convened in Sydney, Australia, in November 2014 for the sixth World Parks Congress. An analysis by Montesino Pouzols *et al.*³, published online during the congress and now on page 383 of this issue, has direct relevance to these ongoing discussions on protected-area policy and practice.

Understanding the performance of protected areas requires clarity on the extent to which biodiversity is represented in them. Gap analysis — an approach designed to assess how well existing protected areas meet conservation goals — was first applied globally as a contribution to the fifth World Parks Congress, held in 2003 (ref. 4). Montesino Pouzols *et al.* have now taken the method to a new level of sophistication, applying state-of-the-art analytical techniques to massive data sets on biodiversity and protected areas. They find that effective delivery of Target 11, despite the small increase in land proportion covered, could triple current levels of protection of terrestrial vertebrate species and ecological regions (sufficient data are not yet available for an equivalent assessment of invertebrate, plant or fungal species, or of freshwater or marine biomes).

Although this is a crucial finding for

global-level policy, it does not directly inform where to fill the gaps in the current protected-area network on the ground. This is because Montesino Pouzols and colleagues use a spatial resolution of 0.2 degrees (equating to squares of about 20 kilometres on each side at the Equator), which, although finer than the resolution of the biodiversity data they analyse, is coarse compared to the resolution of actual protected-area boundaries. However, the authors did run a sensitivity analysis that revealed similar results at spatial resolutions as fine as 1/60 degrees, demonstrating that their aggregate results are robust.

The authors also validate their findings using high-resolution assessments of key biodiversity areas identified through existing national-level analyses for the Philippines, Myanmar and Madagascar. These are sites that significantly contribute to global biodiversity persistence, documented using standard criteria such as those used by the conservation partnership BirdLife International for the identification of Important Bird and Biodiversity Areas or by the Alliance for Zero Extinction for identifying sites that are the single remaining home of one or more highly threatened species⁵. The consultation currently under way to finalize the key biodiversity-area standard was discussed at length at the Sydney World Parks Congress. The authors' validation in turn suggests the way forward to fill protected-area gaps, by using key biodiversity areas as critical inputs for systematic resource allocation and conservation planning. Protected-area coverage of key biodiversity areas is already used⁶ as a marker of progress towards Target 11 and has been proposed⁷ as indicator 87 towards the United Nations' putative Sustainable Development Goal 15.

However, Montesino Pouzols and co-workers also reveal dark clouds on the protected-area horizon. Their modelling shows that projected scenarios for land-use changes that degrade or eliminate habitats and thus preclude protection will foreclose options for efficient achievement of Target 11. What might bring light into this future shadow? Perhaps most important will be to recognize and document protected-area governance and “other effective area-based conservation measures” — which are often currently focused on governmental roles — as including private protected areas and indigenous and community conserved areas⁸. Many key biodiversity areas beyond the current protected-area network are already



Figure 1 | Community protection. The Reserva Natural El Pangán in Colombia is recognized as an Important Bird and Biodiversity Area. Although not designated as a governmental protected area, the Colombian non-governmental organization ProAves works with the local community to safeguard the site, with funding from the Critical Ecosystem Partnership Fund and other international sources — an example of biodiversity protection beyond that afforded by government-designated sites.

PROAVES COLOMBIA