Recasting the Theory of Mosquito-Borne Pathogen Transmission Dynamics and Control

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Mosquito-borne diseases pose some of the greatest challenges in public health, especially in tropical and sub-tropical regions of the world. Efforts to control these diseases have been underpinned by a theoretical framework developed for malaria by Ross and Macdonald (1), including models, metrics for measuring transmission, and theory of control that identifies key vulnerabilities in the transmission cycle. That framework, especially Macdonald's formula for R_0, and its entomological derivative, vectorial capacity, are now used to study dynamics and design interventions for many mosquito-borne diseases. A systematic review of 388 models published between 1970 and 2010 found that the vast majority adopted the Ross-Macdonald assumption of homogeneous transmission in a well-mixed population (2). Studies comparing models and data question these assumptions and point to the capacity to model heterogeneous, focal transmission as the most important but relatively unexplored component in current theory. Fine-scale heterogeneity causes transmission dynamics to be nonlinear, and poses problems for modeling, epidemiology and measurement. Novel mathematical approaches show how heterogeneity arises from the biology and the landscape on which the processes of mosquito biting and pathogen transmission unfold (3). Emerging theory focuses attention on the ecological and social context for mosquito blood feeding, the movement of both hosts and mosquitoes, and the relevant spatial scales for measuring transmission and for modeling dynamics and control.

Mosquito blood feeding and concurrent expectoration creates a wound and a delivery system by which pathogens pass through vertebrate skin to infect vertebrate blood and other target tissues causing diseases such as malaria, dengue, filariasis, Japanese encephalitis, West Nile, Rift Valley fever, and chikungunya. The significant annual health burden of these diseases (4), most notably malaria (5-8) and dengue (9), has raised their profile and increased funding for their research and prevention. The recent global financial crisis meanwhile has increased pressure to show a rapid return on this investment (10). Donors and government agencies must weigh investments in existing public and veterinary health interventions against the development pipeline for vaccines, drugs, diagnostics, and novel mosquito-control technologies, such as new insecticides and genetic interventions. At the same time, policy makers are asking challenging questions about disease control policies, targets for intervention coverage levels, the costs and benefits of combining various interventions, and the optimal ways to scale up regionally or globally. Given the complex, quantitative nature of control targets and policy for mosquito-borne diseases, dynamic models of mosquito-borne pathogen transmission (MBPT) are indispensable tools for investigating these questions (11-14).

Mathematical models of MBPT have been used productively to understand and identify key epidemiological features, to measure transmission intensity, and to guide disease control programs (1, 2). As the need for understanding transmission dynamics and evaluating control options has increased, the types of models being developed and the way they are used have likewise evolved. To understand better the capabilities of current approaches, we recently reviewed the current state of MBPT models (2). Here, we extend that review to critique the models, to look at metrics of transmission, and the way those metrics have been combined with models to better inform and more productively shape disease control policies.

Development of the Models and Metrics

The basic science and accompanying theory for measuring and modeling MBPT developed slowly from 1877, when Manson showed that mosquitoes transmit filarial worms (15, 16). Mosquitoes were then implicated in the transmission of malaria in 1897 (17), yellow fever

98 in 1900 (18), and dengue fever in 1906 (19). Hundreds of pathogen species are now known
99 to be mosquito-transmitted (20), including 38 of clinical significance in humans (21).
100 Throughout that history, mathematical models describing MBPT and control catalyzed the
101 development of concepts and metrics that define the study of mosquito-borne pathogens
102 today (1, 2).

103 The quantitative approach to studying MBPT started with Ronald Ross, who (after showing
104 that mosquitoes transmit malaria) turned his attention to promoting vector control, and to
105 improving malaria diagnostics. He developed a mathematical theory for vector control
106 through larval source management (22) and for MBPT (23, 24), as well as a modeling
107 framework for epidemics in general (1). Ross's transmission models and Alfred Lotka's
108 analysis (25) established solid mathematical foundations for MBPT dynamics (1).

109 As Ross contemplated disease control, he recognized the importance of measuring the
110 intensity of malaria transmission. The proportion of the population with a palpably
111 enlarged spleen – the "spleen rate" – had been a standard measure of endemic malaria even
112 before Laveran made microscopic diagnosis of malaria possible (26). Ross used the
113 prevalence of infection (the proportion of a population found to be infected with malaria
114 parasites by microscopic analysis, called the "malaria rate" or "parasite rate" abbreviated
115 as PR). Driven by a need for more accurate metrics, he developed the "thick film" to
116 improve the sensitivity and specificity of microscopy for diagnosing malaria (1). The use of
117 the PR as a metric consequently increased (26).

118 Ross also devised mathematical formulas relating the force of infection (FOI, he called it the
119 "happenings" rate) to other measurable quantities; *i.e.*, the fraction of a cohort that would
120 be infected over time or at a particular age or in some fixed time period. An important next
121 step came when Muench developed the "reversible catalytic" model into a statistical tool
122 (27) for both infection prevalence and serology by age as measured by the sero-conversion
123 rate (SCR).

124 Ross's mathematical models describing adult mosquito movement and the spatial scales
125 required for effective larval source management (22) helped to motivate and justify mark-
126 release-recapture studies to quantify mosquito movement, which was part of operational

research during construction of the Panama Canal (28). In his books and papers, Ross made the case for developing entomological metrics of the intensity of transmission. In the 1930s, the "infective biting density" was devised (29) to measure the number of infectious bites, per person, per day or per year; it is now commonly known in malarial studies as the entomological inoculation rate (EIR) (30). The original pioneering study also compared the EIR to other metrics of transmission – the PR in older children, and the FOI as it was reflected in the pattern of rising age-specific PR from infancy through childhood. The authors noted that although the patterns were roughly consistent with theoretical predictions, epidemiological measures of transmission were obviously much lower than predicted by entomological metrics (29).

In the 1950s, George Macdonald analyzed and synthesized studies from the previous decades describing the epidemiology of malaria and its vectors in a series of landmark papers (31, 32). His most important achievements are encapsulated in a formula for the basic reproductive number (sometimes called a ratio or rate) for malaria, now called R_0 (Fig. 1) (33-35). Macdonald's formula, which was superficially similar to a threshold criterion developed by Ross, was based on a simple yet compelling mathematical model of the entomological factors associated with transmission, most notably daily mosquito survival (Fig. 1). A component of R_0 is the number of infectious bites that would eventually arise from all the mosquitoes that would be infected after biting a single infectious host on a single day, called the daily reproductive number or vectorial capacity (VC) (36). VC was also affected by the frequency of mosquito feeding on the pathogen's host, mosquito population density relative to host population density, mosquito survival, and the length of the period during which a mosquito is infected but not yet infectious. The basic reproductive number, R_0, describes the expected number of times a pathogen is transmitted from one host to another after one complete pathogen life cycle (Fig. 1). A threshold condition for a pathogen to invade a population is $R_0 > 1$, because each infected host would, on average, have to transmit the pathogen to more than one infected host. As a metric of transmission intensity, R_0 thus encapsulates most aspects of the transmission process, and Macdonald proposed it as a threshold condition for pathogen persistence in

156 the absence of control (34).

157 Macdonald pioneered a quantitative theory of vector control in an era when contact
158 pesticides (*e.g.* DDT for indoor residual spraying) were being used extensively for the first
159 time. Macdonald's analysis was based on a mathematical sensitivity analysis of the formula
160 for R_0 (32), which showed that the potential for transmission was affected by mosquito
161 longevity in two ways: an infected mosquito must survive long enough for the pathogen to
162 mature, and the mosquito must blood feed while infectious, so the longer it lived, the more
163 infectious bites it would deliver. Because the latent period for infections in the mosquito,
164 called the "extrinsic incubation period," is generally longer than most mosquitoes are
165 expected to live (though the length of this period varies depending on the pathogen-
166 mosquito interaction and the environment), the mosquitoes that are most likely to transmit
167 and propagate the pathogen are those that bit an infectious host when they were young
168 and then survived to be quite old (32, 37). More importantly, since mortality affected these
169 two aspects of transmission in Macdonald's model, the potential intensity of transmission
170 would be highly sensitive to mosquito survival. Macdonald's analysis has since been used
171 to advocate for prioritizing modes of control that reduce adult mosquito survival.

172 Macdonald argued that measurement of transmission should become a routine part of the
173 Global Malaria Eradication Programme (GMEP, 1955-1969), and his papers and ideas
174 spawned new research on practical methods for measuring mosquito survival under field
175 conditions, the estimation of R_0, the development of a codified set of methods for
176 estimating the parameters comprising vectorial capacity, and on tests of Macdonald's
177 theory of control (1).

178 By the end of the GMEP, a set of quantities had been identified that were relevant for
179 modeling MBPT dynamics and control along with a set of field metrics and statistical
180 methods for measuring transmission. Transmission could be measured in terms of
181 infection prevalence, exposure to a pathogen either epidemiologically (*i.e.* through the FOI),
182 serologically (*i.e.* through the SCR), entomologically (*i.e.* the EIR), or through the
183 entomological potential (*i.e.*, the vectorial capacity, which can be measured even in the
184 absence of a pathogens). The models made powerful, specific, and testable predictions

about the way these quantities would scale across the spectrum of transmission and likely effects of control, and they set the stage for the study of MBPT through to the present day.

Although the GMEP and a program to eradicate *Aedes* mosquitoes from the New World for yellow fever control were being abandoned, the 1970s were an important transition period in the mathematical study of MBPTs. Important advances came with rigorous applications of the catalytic model to estimate incidence from highly age-stratified PR or serological data (38, 39), and new methods to estimate malaria incidence from longitudinal data (40). The practical issues associated with measuring vectorial capacity spurred more pragmatic approaches for malaria, and in 1980, the WHO returned to using the EIR as a single, comprehensive measure of transmission intensity (30). A new mathematical model was developed for understanding transmission of malaria in highly endemic areas, where immunity was an important feature of the system, and it played a key role in the design and interpretation of a large-scale control trial in Garki, Nigeria (41). The model was later applied to a similar transmission setting in Kenya (42). Studies published between 1965 and 1980 introduced the first simulation models (43, 44) and explored themes of immunity (41), seasonality, spatial dynamics, and heterogeneous mosquito biting and its effects on transmission (45). The state of the science at that time is summarized in several reviews (46-48).

Modern Theory

Research themes introduced during the 1970s have been developed through to the present day. The initial focus on malaria has been expanded to include the broader study of other mosquito-borne pathogens, which are transmitted by vectors with different behaviors and ecologies and which have functionally different transmission dynamics and relations to their hosts. As investment in mosquito-borne pathogen research and interventions has been scaled up, there has been a dramatic increase both in the total number of publications in this field as well as those including theory. At least 388 models that included a mechanistic description of transmission were found in 325 publications between 1970 and 2010 (2); approximately half of these were published after 2005. These models were compared using a detailed, 79-part questionnaire to identify the assumptions they made

about a wide range of biological features considered by the models. Despite the growing body of theory, most models published in the last 40 years bear a striking resemblance to the Ross-Macdonald model (2). Out of 15 core assumptions in the Ross-Macdonald model, most existing models adopted all but one, two, or three of them, leaving most of the underlying framework unquestioned and intact (a detailed description of our methods and findings can be found elsewhere (2)). Does this conservatism reflect the accuracy and appropriateness of the simplifying assumptions required by Ross-Macdonald models, or has the field become canalized to the exclusion of other approaches?

The structure and content of these MBPT models can be understood and classified by the assumptions they make about five distinct components of transmission (Fig. 2): 1) pathogen infection dynamics inside the vertebrate host, including immunity; 2) adult mosquito population dynamics and pathogen infection dynamics inside the mosquito; 3) transmission of the pathogen including the mosquito-host encounter and ensuing blood meal from the mosquito to vertebrate host or *vice versa*, as well as dispersion of the pathogen in infected mosquito or vertebrate hosts; 4) the ecology and population dynamics of immature mosquito population dynamics, involving development from eggs, through four larval instars, pupation and emergence of adults from the aquatic habitats; and 5) egg laying, which links blood feeding adult mosquitoes to immature mosquito populations in both time and space. Not every model of transmission includes every component. Published mechanistic models of pathogen or mosquito population dynamics have generally been developed to address a particular question, so they focus on one or more of these components treating inputs from other components as fixed parameters. A table classifying models by their purpose is also available (2).

These five components have been extended to address specific biological or control questions involving: various modes of vector control (49-51); transmission or disease control with drugs or vaccines (52-55); pathogen evolution and the management of virulence or drug resistance (56); two or more pathogens and facilitation or competition (55, 57); genetic manipulation of mosquitoes or the evolution of insecticide resistance (58, 59); weather or climate and its relative effects on transmission (60); impact of parasite burden and aggregation (61, 62); the role of some specific biological mechanism in

transmission; spatial or metapopulation dynamics (63); and multi-host dynamics (64).

Among the most important innovations in modeling are those that address immuno-epidemiology: models of pathogen population dynamics inside the skin of a vertebrate host, including host immunity and progression from infection to disease (65-67). Different mosquito-borne pathogens interact with their human host in very different ways with important consequences for within-host dynamics: for example compare the microparasitic dynamics of chikungunya (68); interactions among four microparasitic serotypes of dengue (55, 69); the macroparasitic accumulation of filarial worms (61); and the dynamics of superinfection with genotypically and phenotypically diverse malaria parasites (70). Some important consequences of these differences include the relevance of superinfection, the effects of immunity on transmission, and the functional significance of genetic diversity in pathogen populations.

Of great importance for the comparative study of MBPT are functional differences in the immuno-epidemiology of a pathogen-host interaction that constrain the ways transmission can be measured and the sorts of questions that can be addressed for any single disease. Full immunity to filariasis and malaria is not readily developed, and infections persist for long periods of time, so the parasite reservoir in humans is reasonably large. It is thus practical (even if challenging) to measure the prevalence of malaria or filariasis infection in humans and in mosquitoes. Theory suggests that superinfection is an interesting and important metric of transmission for malaria and filariasis, so the study of these parasites has sought methods to measure individual variation in exposure. Because dengue and other arboviral infections cause acute, immunizing infections, the pathogen reservoir is comparatively smaller, and the prevalence of infection in both humans and mosquitoes is much lower. In consequence, individual variation in exposure has received much less attention for arboviral infections, and measures of EIR are more useful for studying malaria, for example, than for dengue. Similar issues affect the comparative ease of studying transmission through the serological status of humans for chikungunya, malaria, dengue, and filariasis. These constraints beg for a comparative approach to MPBT, because even if the vectors differ in some important ways, the observations made from studying pathogen transmission in one system could have great value for understanding the

importance of phenomena that could be important but that can't be measured in the others.

A more recent trend that complements modeling studies is the creation, curation, and analysis of databases describing MBPT, including mosquito bionomics, transmission metrics, and other important variables accumulated over more than a century of investigations (71-74). Mosquito ecology and MBPT are highly heterogeneous over space and time (75-78). At a large scale, it is important to know where transmission is occurring, so maps have played an important historical role in control. The role of maps and the supporting technologies have expanded substantially in recent years with the publication of global maps describing the distribution of malaria (72, 79) and of dengue (9). Also of great interest are databases that have aggregated metrics of transmission, especially those studies that have measured two or more metrics at the same time and place, and that investigated the properties of various metrics across space and time or across transmission intensities (73, 74, 80). The marriage of models and large aggregated databases has made it possible to test the models to an extent that has not been possible before.

Testing Theory

Measuring the different components of vectorial capacity allows the potential intensity of pathogen transmission by any mosquito population to be assessed. But studies adopting this approach have raised important questions about the utility of these: large, poorly quantified errors can arise because of the methods used to catch mosquitoes and estimate bionomic parameters (81); systematic bias in parameter estimates can arise from fluctuations in mosquito populations (82) or senescing mosquito populations, or other assumptions of the underlying models; and in making an estimate of vectorial capacity, errors can be propagated by taking the product of several noisy and potentially biased parameter estimates (83).

Complementary approaches to vectorial capacity involve the indirect estimation of R_0 using other field metrics of exposure, based on the assumptions of a mathematical model (35). Such methods for malaria include the estimation of the EIR, FOI, or PR. A key observation is that the daily EIR is approximately the product of vectorial capacity and the

302 *net infectiousness* of the pathogen reservoir in the vertebrate hosts, *i.e.* the probability a
303 mosquito becomes infected after feeding on the pathogen's vertebrate host (1, 41). This
304 makes it possible, at least in theory, to measure vectorial capacity in two different ways
305 (assuming there is some independent estimate of net infectiousness). The Ross-Macdonald
306 model and most models developed in this tradition assume the FOI is the product of the
307 EIR and the efficiency of transmission per bite, and the relationship between the EIR and
308 the PR is given by simple formulas. These can be tested against the observed values. Other
309 measures include estimating the FOI from changes in serology in a population *versus* age or
310 time (84, 85). For dengue and other acute immunizing infections in simple systems, R_0 can
311 be measured by monitoring changes in the number of cases over time (86). Measuring
312 changes in the number of cases becomes more difficult for some pathogens that are passed
313 among many mosquito or many vertebrate host species, especially when the epidemiology
314 of the pathogen and presentation of the disease differs for each species. Measuring changes
315 in the number of cases is also difficult for the largely endemic diseases of malaria and
316 filariasis (35). Filariasis models focus on the accumulation of worm burdens, and malaria
317 epidemics are restricted to areas with unstable transmission or populations encountering
318 malaria for the first time.

319 The richness of methods for estimating R_0 provide different ways of cross-validating or
320 "testing" the underlying theory, and unsurprisingly, such studies have also exposed some of
321 the weaknesses due to the simplifying assumptions of the Ross-Macdonald model. Early
322 tests of the theory for malaria that compared estimates of R_0 based on the EIR and FOI,
323 showed large discrepancies because transmission of malaria parasites from mosquitoes to
324 humans was highly inefficient (87) – many infectious bites are required for each infection,
325 which implies a high ratio of EIR to FOI – which is similar to what Macdonald found in his
326 reanalysis of earlier studies (31). Similarly, early studies of filariasis independently
327 concluded that transmission is more inefficient than typically assumed (88). Further
328 studies of malaria using an aggregated dataset of paired transmission metrics detected a
329 strongly non-linear, empirical relationship that exists between the EIR and the FOI,
330 including ten- to hundred-fold quantitative discrepancies in places with the highest

measured transmission (73).

Published estimates of R_0 for mosquito-borne pathogens are among the highest recorded across all pathogens (34, 35, 89). At first glance, these predictions seem reasonable given the potential for extraordinarily high mosquito population densities and biting rates, but upon more careful examination, and in light of the observed inefficiencies in transmission, they are questionable. Also, the highest estimates are generally based on entomological metrics (*i.e.*, EIR or vectorial capacity), which are not directly comparable to those collected for directly transmitted diseases. Where non-entomological estimates have been made, which are generally measured using methods that can be compared to estimates made for other pathogens, the estimates obtained are much lower (35, 90). The extremely high estimates of R_0 obtained from calculations involving vectorial capacity are due to the implicit assumption that across the spectrum of intensity, the number of infections is proportional to the number of infectious bites.

Heterogeneous biting, a name for the empirical fact that a small fraction of the vertebrate population tends to supply most of the blood meals for mosquitoes, is one factor that could explain what appears to be inefficient transmission because infectious mosquito bites are redistributed in a way that tends to reduce the number of unique individuals who would be infected (34, 73, 80, 88, 91). Efficiency in transmission also declines if there are only a few vertebrate hosts in the neighborhood who could be infected. Some models of heterogeneous biting have become integrated into the standard Ross-Macdonald model (34), but much less work has been done on the spatial scales of transmission and the effects of local mixing between human and mosquito hosts.

Critiquing Theory

Despite the enormous and expanding body of evidence and theory describing MBPT dynamics and control, highly inefficient transmission challenges the applicability of the basic theory. These same questions emerge from attempts to use maps and models together. How heterogeneous is transmission over time and space? What factors give rise to heterogeneous transmission? What are the appropriate scales for modeling MBPT

dynamics and control? What are the appropriate sampling frames for measuring transmission?

Heterogeneity in transmission is observed at every spatial scale (Fig. 3). At small scales (*e.g. < 100* meters), where mosquito and human behavior and ecology give rise to heterogeneous biting, there are important questions about how mosquito vectors and hosts are distributed across the landscape, how this influences where transmission occurs and how an increased understanding of those processes can be applied to improve efforts to model transmission and apply the lessons to reduce disease. Heterogeneity is also important at spatial scales ranging from kilometers to continents, where ecology and biogeography determine the composition and dynamics of the vector and host communities and the intensity of transmission. An important unanswered question is how the same processes give rise to such a diverse set of patterns across different scales.

The Ross-Macdonald model provides a starting point for dealing with such questions, but it also has limitations. Among the most widely adopted simplifying assumptions of the Ross-Macdonald model was mass-action, a 19th century principle from chemistry describing the reaction rates of molecules in an ideal solution. The Ross-Macdonald model assumes that all hosts are identical and equally exposed to pathogens at the same rates, and that the probability of transmission is proportional to the product of host and vector densities. Thus, regardless of the size of the population, there are no epidemiologically important correlations in the distribution of consecutive bites on the same or different hosts. By assuming mass-action it is possible to reduce a great deal of complexity and arrive at a relatively simple expression for R_0.

Macdonald's formula for R_0 is appealing, in part, because it serves several mathematical purposes at once. It is the expected number of secondary infections arising from an initial infection in a non-immune population, and so it gives a deterministic threshold for the pathogen to establish endemic transmission chains. It also provides a single metric of the intensity of transmission that is suitable for comparing the transmission reducing effects of different modes of control, either alone or in combination. The effects of any mode of control on transmission can be compared with the effects of modes of control that reduce

388 adult mosquito population density, which is linearly proportional to R_0. Depending on the
389 patterns of contact, however, the simple scaling relationships that make all these
390 interpretations alike could change because of factors that were omitted from Macdonald's
391 formula.

392 Pathogen transmission by mosquitoes has been characterized as being highly local and
393 focal, with transmission foci and hotspots (76). Hotspots are affected by the juxtaposition
394 of the aquatic habitats suitable for the development of immature mosquito populations to
395 the locations where blood feeding occurs, and by a range of mitigating factors. All
396 transmission involves pathogen movement in either moving infected mosquitoes or
397 moving infected hosts, but what factors determine the size of a focus or the scales that
398 characterize transmission? Ironically, though Ross's first model addressed questions about
399 local mosquito movement (22), movement and pathogen dispersal have not become a core
400 part of MBPT theory.

401 If local processes drive transmission, then the spatial scales that characterize transmission
402 will tend to be small. In simple systems with one host and one vector, effective host
403 population sizes must be small, so that infectious bites are distributed on only a few hosts.
404 In more complex systems, notably zoonotic mosquito-borne pathogens with many vectors
405 and many hosts, transmission patterns are affected by the diversity of less-competent or
406 non-competent hosts (92). The more heterogeneous the distribution of bites on those few
407 hosts, the greater the number of bites that would land on the same few hosts, and the lower
408 the expected number of different hosts who would become infected. Because of local
409 mixing and heterogeneous biting, the actual number of new cases arising from an index
410 case is thus strongly limited by the number of hosts that could possibly be bitten. The
411 difference between the number of infectious bites and the number of infections is due to
412 repeated transmission of pathogens to the same few hosts thereby dampening
413 amplification. In more mathematical terms R_0 must be a non-linear function of vectorial
414 capacity. The functions describing that relationship depend on the distributions of hosts
415 and vectors and the spatial scales that characterize transmission.

416 Vectorial capacity counts the number of infectious bites arising from a single host on a

417 single day. The formula originally assumed hosts were perfectly infectious, but the formula
418 has also been modified to include vector competence. It does not take into account the
419 redistribution of infectious bites on a finite number of vertebrate hosts in a population with
420 heterogeneous exposure. The problem with inferring transmission by counting infectious
421 bites arising is illustrated by analogy: if R_0 for directly transmitted pathogens were
422 proportional to the number of inocula shed, and by assuming each one of those particles
423 reached and infected a different host, the estimates for other diseases would likely be just
424 as high as for indirectly transmitted mosquito-borne pathogens. What the concept of
425 vectorial capacity does not account for is the potentially complicated patterns of human-
426 mosquito contact in space and time that distributes infectious bites among a cascade of
427 different hosts with varying infectious status, immune level and innate susceptibility. Just
428 as some inocula are redundant in infecting the same susceptible host many times over, so
429 too are bites by infectious mosquitoes redundant whenever transmission is localized or
430 intense.

431 Mathematical theory has explored the properties of spatially localized transmission,
432 including the consequences for transmission of heterogeneous biting (34, 45, 47, 93-96),
433 local spatial heterogeneity (94, 96), metapopulation dynamics (63), and small population
434 sizes (34, 96, 97). Other frameworks have been developed more recently that show how
435 heterogeneous transmission arises and these lay the foundations for a systematic study of
436 the way these factors vary across systems (3, 92).

437 Despite highly spatially heterogeneous patterns of transmission, mathematical methods
438 continue to use R_0 as a deterministic threshold for the ability of a pathogen to invade a
439 system, *i.e.*, if $R_0 > 1$ then a pathogen will tend to spread. Heterogeneity of all kinds calls
440 into question the value of using a single number to describe how well a pathogen invades.
441 Expressions for R_0, even with heterogeneity, describe how spread would eventually occur,
442 *i.e.*, the asymptotic behavior of the system, without regard to transient phenomena. Such
443 transients are particularly important during invasion if pathogen establishment is
444 stochastic. If the underlying biological determinants of vectorial capacity are spatially and
445 temporally heterogeneous, then the *expected outcome* will be expected to vary in some way

446 over space and time. The focal nature of transmission raises questions about the relevance
447 of R_0 as a threshold for determining whether the pathogen would tend to invade *here* and
448 *now* even if the threshold has determined that it could invade *somewhere* or *sometime*.
449 Because invasion is a stochastic phenomenon, it matters where and when the pathogen is
450 introduced and what is the *local* vectorial capacity (94, 96). To put it another way, it may be
451 possible for a pathogen to invade a potential hotspot, but only if it happens to find it. In this
452 context, it is important to note that there is no mathematical construct for defining a
453 "hotspots" based on dynamical criteria.

454 ## Recasting Theory

455 Development of theory and tests of that theory have raised questions about how actual
456 transmission differs from mass action, and how heterogeneity and poor mixing affect
457 quantitative conclusions about control. Ideas from the Ross-Macdonald model, such as the
458 calculation of thresholds and the sensitivity of transmission to adult mosquito longevity,
459 have been useful. Questions confronting contemporary policy for mosquito-borne
460 pathogens concern quantities describing phenomena that vary through time and space and
461 at different scales.

462 In order to address these questions we believe new theory should be based on the events
463 that give rise to transmission and accommodate extensive variation in time and space. New
464 models of transmission process should emerge from a quantitative description of the
465 complex local biological interactions among vectors and their hosts. The logic that
466 motivated Macdonald's formula for R_0 is compelling, and it seems likely that any attempt
467 to develop a quantitative index of transmission would adopt many of the same set of
468 parsimonious assumptions. On the other hand, we argue that estimates of R_0 would be
469 more useful if they accounted for the spatial and temporal dimensions of transmission and
470 the way transmission arises from an ecological context and mosquito blood feeding
471 behavior.

472 An alternative way of understanding the ecology of MBPT, articulated by Hackett for
473 malaria, is to assume that local transmission is a complex puzzle that is, like chess, built up

474 from a few simple pieces (98). Following Hackett's logic, Najera *et al.* proposed an
475 alternative theory of malaria control based on ecological or social contexts giving rise to
476 malaria transmission (99). They discussed six specific ecological settings: the African
477 savanna, plains and valleys outside Africa, forest and forest fringe areas, highland fringe
478 and desert fringe, seashore and coastal malaria, and urban malaria. Four specific patterns
479 associated with occupations or social conditions were agricultural colonization of jungle
480 areas, gold and gem mining, migrant agricultural labor, and displaced populations.
481 Macdonald similarly found a categorical approach useful when he proposed three
482 categories of transmission: stable, unstable, and epidemic (33). Macdonald was as
483 interested in endemic malaria (33) as well as epidemics (100), but what set his approach
484 apart was the development and application of a quantitative theory based on R_0 to
485 understand both kinds of phenomena. Could the rigor of Macdonald's quantitative
486 approach be applied to codify these categories for malaria, to identify some useful set of
487 categories for mosquito-borne pathogens of humans, or of complex transmission dynamics
488 of pathogens with many mosquito and vertebrate animal hosts? If so, how does
489 transmission in these ecological settings differ in ways that are not captured by R_0?

490 One way to fuse the quantitative methodology of the Ross-Macdonald model with the
491 qualitative view adopted by Hackett and others is to build models that identify the basic
492 components, which will likely include many parts of the formula for vectorial capacity.
493 What merits more attention is a systematic way of looking at the way complexity arises
494 from the way the pieces fit together. The fundamental questions are about heterogeneity in
495 transmission and the biology that underlies highly local and focal transmission; *i.e.*, poorly
496 mixed populations. Just as the theory of sexually transmitted pathogens successfully recast
497 itself around the concept of heterogeneity in numbers of sexual partners and sexual contact
498 networks in network models, so too must the mathematical theory for mosquito-borne
499 pathogens recast itself around the underlying biology if we are to understand and quantify
500 how ecological and social contexts affect MBPT dynamics and disease control.

501 A useful concept around which the theory of MBPT can be recast is that of key
502 epidemiological encounters (Fig. 3). It is well known that the key encounter for mosquito-

503 borne pathogens is the blood meal, but the spatial context for these encounters has not
504 been carefully examined mathematically. The number, timing, and intensity of encounters
505 are largely a function of how many mosquitoes emerge from aquatic environments located
506 near areas where hosts spend time. The dynamics of larval mosquitoes in aquatic
507 environments are complex and poorly understood, depending on habitat selection by egg-
508 laying adults, biotic and abiotic drivers of developmental success, and how and the extent
509 to which density-dependent mortality operates. Following emergence from these
510 environments, adult female mosquitoes undergo flights for nectar feeding and mating and
511 then an appetitive search to find a blood meal host, a short flight laden with blood to find a
512 place to rest, a search to find a suitable aquatic habitat for egg laying, and then a repeated
513 appetitive quest to find another blood meal host (101). Given that the mobility of
514 mosquitoes is on average somewhat limited, locations where blood feeding occurs must be
515 close to other resources such as aquatic habitat and resting sites. Mosquitoes may exercise
516 choice among locations for host seeking and among individual hosts (102) for blood
517 feeding based on their attributes, including CO_2 emission, odors (103), body size (104,
518 105), type of clothing worn, and other factors including elevation, the overall diversity of
519 the vertebrate host community (92), and home, nest, or habitat type. It is also important to
520 bear in mind that hosts are also heterogeneously distributed in the environment and are
521 moving targets (106), and that hosts can exhibit defensive or avoidance behavior, possibly
522 in response to increased biting by mosquitoes (107). The risk of hosts being bitten is a
523 function of where and at what time of day they frequent locations in which mosquitoes are
524 searching for blood meals.

525 Mosquito biology including the search for egg-laying sites and blood feeding strategies thus
526 emerge as important elements in a new theory that affect transmission as much as blood
527 feeding behavior. Mosquito strategies can range from active questing at night over fairly
528 long distances, such as by *Culex* in agro-ecosystems, to stationary ambush feeding where
529 species such as *Ae. aegypti* or *Ae*. *albopictus* wait in protected areas until the host arrives.
530 Similarly, the patterns of human activity and mobility in relation to these vector search and
531 feeding strategies are of great importance for understanding transmission. Recent evidence
532 suggests that human social networks are just as important for transmission within cities as

mosquito ecology (108), and that movement networks are a critical element of transmission within and among countries (109, 110). Similar problems arise in the study of complex transmission dynamics involving many vectors and many vertebrate hosts where contact networks must contend with the problems of territoriality, seasonal migration, aggregation around resources, and group social structure. In addition to defining the context for key encounters, movement of mosquitoes and hosts at times when mosquitoes are actively feeding jointly govern how pathogens spread during an outbreak and persist over time. There is an urgent need to improve the methods for using data describing mosquito and vertebrate host mobility to understand pathogen transmission dynamics and persistence across scales for pathogens as different as chikungunya, dengue, malaria, and filariasis.

A closely related core concern is that statistical theory must also be developed to inform the spatial scales at which the metrics can be used to estimate transmission in models or to define appropriate sampling frames. The methodology used to analyze transmission metrics has improved substantially since 1970, but like transmission models, there has been very little progress in the basic metrology or in relating those metrics to transmission or control. In particular, the metrics themselves have been poorly validated, and the sampling properties of the metrics (*i.e.*, bias and measurement errors) remain poorly defined.

Concerns about the statistical properties of the metrics are not just hypothetical. The processes of setting coverage targets to meet national goals, of evaluating the impact of mass interventions, of designing trials for interventions that reduce transmission, or of understanding transmission rely on data describing the intensity and scale of transmission. The challenge is that transmission of mosquito-borne pathogens is likely heterogeneous at every scale. In such an environment, what is the appropriate sampling frame for measuring transmission? Having a good metric is often the rate-limiting step for inference, so the practical way forward is to develop theory around the metrics. What windows of space and time are valid for the selected metrics?

If dispersion and the number of hosts in the neighborhood limits transmission, rather than

562 vectorial capacity, then thresholds on the coverage of vaccines, drugs, and other host-based
563 interventions may not scale linearly with vectorial capacity. What remains unknown, and is
564 highly relevant for understanding transmission dynamics, is what happens to transmission
565 as locally available hosts become saturated. It may be that, despite the nonlinearities in
566 transmission caused by heterogeneous biting and local transmission, vectorial-capacity-
567 based estimates of R_0 are still relevant in an analysis of vector-based coverage levels and
568 thresholds to eliminate a pathogen from an area. What may also be true is that the
569 thresholds may scale differently for different modes of control depending on the context.
570 What is needed now is a new approach to measuring and modeling these aspects of
571 transmission that can lay the foundations for an improved understanding of MBPT
572 dynamics and control.

573 ## Conclusions

574 The Ross-Macdonald theory established a critically important framework for the study of
575 infectious diseases, and it has matured substantially over the past century. The central idea
576 is based on the notion of transmission intensity, which is implicit in Macdonald's formula
577 for R_0. There are good reasons to continue to use this approach, while also carefully
578 questioning its many simplifying assumptions. The question is not whether R_0 and
579 accompanying theory is wrong. All models make simplifying assumptions, all scientific
580 inference is based on some kind of model (*i.e.*, including statistical models and all kinds of
581 conceptual models), and simple models are often exceedingly useful. The issue is whether
582 the omission of certain biological features undermines the application of the model. In this
583 case, does including heterogeneous transmission improve conclusions based on R_0 and
584 predictions about the effective control of mosquito-borne diseases?

585 The observation that most heterogeneity in transmission shares a common spatial
586 dimension begs for the development of a spatially rich theory that can accommodate the
587 limited movement of individual mosquitoes and hosts in variable and sparsely or densely
588 populated landscapes. Movement is especially critical for arboviruses and other strongly
589 immunizing infections where host populations become progressively immune and the

number of susceptible hosts can be depleted. Similar issues will likely affect other pathogens, as well. General theory, however, remains tethered to the core assumptions and non-spatial structure of the Ross-Macdonald model.

Analytical insights from theory developed for directly transmitted pathogens may be required to guide the development of detailed simulations, to identify priorities for field research, and ultimately to guide the design of policy. The seeds of the new generation of theory that we call for have been sown by models of mosquito-borne pathogens (3, 34, 45, 47, 63, 92-96), but the continued development, investigation, and widespread adoption of such approaches and connection with the underlying biology have not yet been fully realized. Advances in theory developed for directly transmitted pathogens, including theory describing poor mixing and networks, have not yet been incorporated into the theory for mosquito-borne pathogens. The concepts of networks and social distance have long been ignored, but there is now evidence of their importance for MBPT (108). Development of a rich theoretical perspective on networks, motivated by the biology of mosquitoes and their hosts, would be a valuable addition to mosquito-borne pathogen theory.

The success of any new theory will be measured by its utility in specific contexts and by its ability to inform decisions weighing the impacts of various modes of control against their costs. Ross-Macdonald theory provides specific advice about the likely effects of drugs, vaccines, and mosquito control on pathogen transmission, and Macdonald's formula for R_0 is highly compelling and frequently used. On the other hand, it is difficult to place confidence in this kind of advice when tests of the theory continue to expose inadequacies. Should such a theory be used to determine how finite global resources are allocated? For example, should resources be diverted to contain artemisinin-resistant *Plasmodium falciparum* before it spreads beyond Southeast Asia? How should resources be reallocated in light of knowledge of the distribution of pyrethroid-resistant *Anopheles gambiae* in Africa and elsewhere? How could a new vaccine against malaria or dengue be most effectively deployed, and should resources be diverted from existing mosquito control programs to do so? Is pathogen elimination the optimal strategy for a country, and if so, on

what time frame? How can limited resources be best used to detect and respond to an introduced exotic pathogen (*e.g.*, Rift Valley fever virus)? Some sort of model will be used to answer all of these questions, but only models that address the unexplored topics identified herein can accurately weigh costs against benefits across different scales of transmission intensity and levels of investment. No single approach is likely to be optimal for every question, so a hierarchy of models and modeling approaches is needed to identify priorities, which will subsequently require empirical validation. Given the inherent uncertainties, the best way to achieve a robust policy recommendation is through the comparison of multiple, independently derived models.

Advancing the theory of mosquito-borne pathogen transmission requires a new synthesis that realistically acknowledges the ecological context of mosquito blood feeding and its quantitative impact on transmission. Specific objectives should be to develop new models that provide guidance about which details are most relevant for increased understanding of transmission dynamics and what types of interdisciplinary collaborations are necessary to make those advancements. These must be rigorously linked to field studies and extensive data on transmission metrics that has already been generated, but there is also a need to develop new theory exploring mosquito ecology and behavior, mosquito and vertebrate host movement, spatial heterogeneity in complex epidemiological landscapes, and the way those factors lead to key epidemiological encounters. These are among the most promising frontiers with potential for high impact in mosquito-borne disease modeling research and its application in disease prevention.

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DLS, TAP, RCR, CMB, TN, LFC, AME, DBG, AL, & JRCP conceived the study. DLS, TAP, RCR, CMB and TWS designed the study protocol. DLS, TAP, RCR, CMB, TN, LFC, AME, DBG, AL, JRCP, DB, CB, CC, DATC, AJG, MLG, PWG, DMH, GH, EYK EM ALL, DMP, WKR, NR, BKS, AJT, and TWS implemented the study. DLS, TWS, and JS designed the figures. All authors contributed to writing the manuscript, but DLS, TAP, RCR, CMB, UK, HCJG, JMC, WKR, SIH, TWS played a major role. All authors saw and approved the final draft. DLS, TAP, RCR, SIH, and TWS critically revised the manuscript for intellectual content. DLS is guarantor of the paper.

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