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Revising ecological assumptions about Human Papillomavirus interactions and type replacement

Short Title:

Revising HPV ecology

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Abstract

The controversy over whether vaccine-targeted HPV types will be replaced by other oncogenic, non-vaccine-targeted types remains unresolved. This is in part because little is known about the ecology of HPV types. Patient data has been interpreted to suggest independence or facilitative interactions between types and therefore replacement is believed to be unlikely. With a novel mathematical model, we investigated which HPV type interactions and their immune responses gave qualitatively similar patterns frequently observed in patients. To assess the possibility of type replacement, vaccination was added to see if non-vaccine-targeted types increased their 'niche'. Our model predicts that independence and facilitation are not necessary for the coexistence of types inside hosts, especially given the patchy nature of HPV infection. In fact, independence and facilitation inadequately represented co-infected patients. We found that some form of competition is likely in natural co-infections. Hence, non-vaccine-targeted types that are not cross-reactive with the vaccine could spread to more patches and can increase their viral load in vaccinated hosts. The degree to which this happens will depend on replication and patch colonization rates. Our results suggest that independence between types could be a fallacy, and so without conclusively untangling HPV within-host ecology, type replacement remains theoretically viable. More ecological thinking is needed in future studies.

Keywords: HPV, within-host ecology, strain replacement, strain interactions, metapopulation dynamics.

Introduction

Infection by Human Papillomavirus (HPV) is responsible for approximately 270,000 cervical cancer deaths and roughly 97,000 cases of other cancers (e.g. anal, oropharyngeal) globally every year (Tota et al., 2011b). The significance of finding a virus as a causal agent to cancer cannot be understated since it permits us to prevent cancers with vaccines. Two vaccines, Cervarix ® and Gardasil ®, are used to prevent cancer by the two most common oncogenic high-risk (HR) HPV types, namely, HPV-16 and -18. Controversy has surfaced around the strain specificity of the HPV vaccines, since other strain-specific vaccines have led to strain replacement (reviews (Gandon and Day, 2008; Martcheva et al., 2008)), such that strains not targeted by the vaccine increase in prevalence over time. Thus, the removal of these vaccine-targeted types (vaccine types) could lead to an increase of other HR types not targeted by the vaccine (non-vaccine types). Alarmingly, a recent increase in prevalence of non-vaccine types was measured in vaccinated young women and in the study population (Kahn et al., 2012) -- a potential first warning that type replacement in HPV is occurring.

Whether a population will expand its niche once another population is removed from a shared environment is fundamentally an ecological question. Indeed, predicting the outcome of removing the vaccine types, HPV-16 and -18, first requires understanding how HPV types interact ecologically during co-infections. In fact, untangling HPV type interactions could also help us understand disease progression (Spinillo et al., 2009). Yet, despite these important reasons, little is known about HPV type interactions and ecology. Here, we analyze this problem using an ecological framework and we consider the impact the vaccine has on the within-host ecology of HPV.

The only type-type interaction that has been clearly demonstrated is that some types interact via the immune response. Types phylogenetically related to HPV-16 (i.e. types in the species α-9) have a negative effect on its viral load (Williams et al., 2002; Xi et al., 2009). Likewise, types with similar epitopes on the vaccine-targeted capsid protein, L1, to those of the vaccine-types HPV-16 and -18 (Christensen and Bounds, 2010) experience some cross-protection by the vaccine (HPV-31, -33, -45, -51 (Wheeler et al., 2012)). Together these studies demonstrate 'immune-mediated apparent competition' (Mideo, 2009) between some related types.

Of all HPV infections 30-50% are multiple infections and, concurrent acquisition (presumably due to co-transmission) of various types is common (Plummer et al., 2011; Thomas et al., 2001). How such a large diversity of HPV types can regularly coexist inside hosts is not understood and has led to speculations of facilitative or 'synergistic' interactions between types (Elbasha and Galvani, 2005; Woodman et al., 2007) or that types are independent (Plummer et al., 2007; Stanley et al., 2006). In contrast, there is some evidence that HPV types may compete for resources (McLaughlin-Drubin and Meyers, 2004), either by co-infecting the same cells and competing for intra-cellular resources or by competing for cells via blocking cell entry. Overall, then, the picture as to how HPV types interact inside hosts is not clear. Clarifying how types interact should have predictive power. Two prior mathematical transmission models (Elbasha and Galvani, 2005; Poolman et al., 2008) found that the occurrence of type replacement will depend on whether types compete or facilitate, and so, their results hinge on the assumptions about within-host interactions. However, the within-host interactions were a black-box in these models, and they continue to be so today.

Based on common interpretations of epidemiological data, there are two main hypotheses of how HPV types interact: facilitation or independence. Support for the former comes from

studies that have found that seropositive patients are more likely to become seropositive with another type (Dillner et al., 2010). The latter is supported by several studies that have not found patterns of clustering at the epidemiological level (reviews (Dillner et al., 2010; Tota et al., 2013)). It is reasoned that the random distribution of types at the population level implies that within hosts there is 'no competition' or 'no interactions' (Garnett and Waddell, 2000; Tota et al., 2013) and that competition between types should be detectable epidemiologically because types that compete would not be found together in co-infections (Kaasila et al., 2009; Palmroth et al., 2012). Underlying this is the concept of 'superinfection' (Levin and Pimentel, 1981; Nowak and May, 1994), where a host already infected by one strain can become infected by another but due to strong within-host competition the new strain quickly excludes the other, thus implying that strains cannot coexistence inside hosts, i.e. co-infections are not possible. Given that independence is the most accepted hypothesis, it is believed that the vaccines will not affect non-vaccine types (Schiller and Lowy, 2012).

Here we test these hypothesized interactions by investigating which interaction scenario behaves most like HPV co-infections. Using a within-host model we investigated independence, facilitation, resource competition (a form of competition that has been largely ignored) and their combinations. This within-host approach allows us to look inside the black-box by explicitly considering the behaviour of different possible interactions inside unvaccinated and vaccinated hosts.

We found that within-host ecological interactions that are not solely independent or facilitative can readily give rise to observed co-infection dynamics. Hence, we caution that the current interpretations of epidemiological data require more support.

Methods

Model

HPV is a small double stranded DNA virus that infects epithelial cells. As a non-lytic virus, HPV's replication cycle is linked to the life cycle of the host cell, meaning that HPV must infect basal epithelial cells (Kines et al., 2009; Schiller et al., 2010) and follow them up through the epithelial column until they die naturally at the surface of the skin (Doorbar et al., 2012). Therefore, new virions are not released until the cells die at the surface (Fig. 1a .i) and HPV needs abrasions in the skin to reach and infect new basal epithelial cells (Fig. 1a .ii)(Doorbar, 2005; Doorbar et al., 2012). This spatial restriction implies that HPV infections are localized, which leads to characteristic lesions, or warts (Doorbar, 2005). HPV infection should thus be conceptualized as occurring in various "patches" distributed across space (Fig. 1a).

We developed a novel patch model that represents HPV co-infections that is based on the Levins metapopulation models from ecology (Levins, 1969), which is a useful framework for understanding plant population dispersion and interactions (Husband and Barrett, 1996). These models describe patches as areas where plants are immobilized and seeds are carried by wind or pollinators to new patches. Here, patches are localized areas of the epithelium that are infected by one or multiple HPV types and that produce free virions (analogous to seeds). Note that if they are infected with HR types these patches become lesions over time.

Apart from patches, our model also includes an explicit cellular immune response and an implicit humoral response. A cellular immune response, i.e. an infiltration of cytotoxic T-cells (CTL) is needed to clear lesions (Stanley, 2006). The equations of our model represent the population of CTL, Z, the patches infected with HPV-16, P_{16}, patches with another HR non-vaccine type, P_{hr}, co-infected patches, P_{co}, and the proportion of empty patches, P_o. The model, which is graphically represented in Fig. 1b, is

$$
\begin{aligned}
\frac{dZ}{dt} &= \gamma(P_i + P_{co})Z - \mu Z \\
\frac{dP_{16}}{dt} &= \frac{e}{w_{16}}(f_{16}P_{16} + f_{16co}P_{co})P_0 - \frac{\varepsilon}{w_{hr}}(f_{hr}P_{hr} + f_{hrco}P_{co})P_{16} - \alpha_{16}P_{16}Z \\
\frac{dP_{hr}}{dt} &= \frac{e}{w_{hr}}(f_{hr}P_{hr} + f_{hrco}P_{co})P_0 - \frac{e}{w_{16}}(f_{16}P_{16} + f_{16co}P_{co})P_{hr} - \alpha_{hr}P_{hr}Z \\
\frac{dP_{co}}{dt} &= \frac{e}{w_{16}}(f_{16}P_{16} + f_{16co}P_{co})P_{hr} + \frac{\varepsilon}{w_{hr}}(f_{hr}P_{hr} + f_{hrco}P_{co})P_{16} - (\alpha_{16} + \alpha_{hr})P_{co}Z \\
P_0 &= 1 - P_{16} - P_{hr} - P_{co}
\end{aligned}
\tag{1}
$$

where the *i* represents *16* or *hr* depending on the case in question, i.e. which type has the CTL mounted against. Here, CTL proliferate at rate γ, die at rate μ, and clear a patch with type *i* at rate α_i. Singly infected patches produce virions of type *i* at rate f_i, while co-infected patches do so at a rate f_{ico}. The establishment rate of the non-vaccine type into patches already with HPV-16, ε, can be different from the rest of the establishment rates, *e*. The humoral response (via neutralizing antibodies) decreases the ability of free virions to infect new patches. Therefore, we approximate this effect by decreasing the establishment of patches, *e* and ε, by dividing them by the parameters w_{hr} or w_{16}. See Appendix A for derivations and other details, such as how we adjusted this model to represent different interaction scenarios.

This patch approach is representative of HPV infections that infect layered squamous cell epithelium, regardless of location in the body. However, this framework does not apply to HPV infections of transformation zones where the basal cells are exposed at the surface. For these, a homogeneous mixing model of individual cells might be more suitable. Also note that this model assumes re-entry of virions and not infection by a new contact episode. We believe that when an abrasion is formed, re-entry is more likely than infection by a new sexual contact event given that virions on the surface are more likely to be from the infected cells then from a one-time inoculum.

Model Parameterization

We obtained estimates for CTL parameters from the biomedical literature (μ and γ from (de Boer et al., 2001)). CTL killing rates specific for HPV were not found in the literature, and therefore, the killing rate, α, was varied between 0 and 0.5 day^{-1} which is consistent with estimates by (Asquith et al., 2006) and is under the lower bound of (Yates et al., 2007). CTL killing rates of HPV infected cells should theoretically be slower than those measured in HIV infections because of limited access to cells inside the epithelial layers. Given the novelty of this model and the lack of HPV kinetics studies, we could not obtain point estimates for other parameters, so we created plausible ranges consistent with the known natural history of HPV (Table A.1).

Analysis: Interaction scenarios

To investigate competing hypotheses, parameters were varied to represent possible combinations of interactions. To consider resource competition, we included co-infected patches where types interact by infecting the same cells and thus have a reciprocal negative effect on each other's replication rates ($f_{16co} < f_{16}$ and/or $f_{hrco} < f_{hr}$). In the case of no intra-patch competition both co-infected patches and singly infected patches produced virions at the same rate ($f_{16co} = f_{16}$ and $f_{hrco} = f_{hr}$). The proposed mechanism for facilitation is that previous infection of one type allows another to establish in the same part of the epithelium. Therefore, to include facilitation we varied the establishment rate, ε. In the no facilitation scenario, the establishment rate into patches with HPV-16, ε, was set to equal that into patches with the same

type ($\varepsilon = e = 1$). To consider independence, we used model A.3 (Appendix) which does not have competition for patches, intra-patch competition or facilitation. Finally, we also considered both competition and facilitation together and, in the vaccinated case, competition only for patches (model A.2, Appendix).

Analysis: Immunity scenarios

Natural immunity

HPV is a very poor immunogen, so we assumed the adaptive response was absent during the first 10 months of infection (Stanley, 2006), and then it attacked either HPV-16 (equation Z grows with respect to P_{16} and $\alpha_{16} > \alpha_{hr}$) or the other type first (equation Z grows with respect to P_{hr} and $\alpha_{hr} > \alpha_{16}$). Cross-reactivity was varied from none ($\alpha_{hr} = 0$ or $\alpha_{16} = 0$) to full cross-immunity. Since antibodies play little to no role in natural immunity against HPV (many hosts during natural HPV infection do not seroconvert (Baseman and Koutsky, 2005; Carter et al., 2011)) we set $w_{hr} = w_{16} = 1$ for the natural cases. With the natural cases we asked: which ecological scenario best represented natural co-infections?**Vaccine immunity**

To represent vaccine immunity, the proliferation of CTL is only linked to the vaccine-type, HPV-16. Therefore, the Z equation is $\frac{dZ}{dt} = \gamma(P_{16} + P_{co})Z - \mu Z$.

The vaccine induces a strong humoral immunity response (up to 100 fold the natural antibody response (Schiller et al., 2008)), therefore we set w_{16} to be 100. Since the virus-like-particles (VLP) used in the vaccine are very immunogenic, they effectively induce several immunological pathways, which includes initiating helper T-cell responses (Stanley, 2010). Thus, the vaccine has been shown to generate a cell-mediated response to the vaccine-targeted protein, L1, and this

response can also be cross-reactive with non-vaccine types (Christensen and Bounds, 2010; Emeny et al., 2002; Pinto et al., 2006; Weinberg et al., 2012). We assumed the CTL invaded 28 days post infection. Since no empirical estimate was available for the timing of the onset of the vaccine response, we estimated this by considering the slow nature of HPV′s non-lytic replication cycle. Squamous epithelial cells require 3 weeks to complete their life cycle (Stanley et al., 2006), therefore, we assumed it took at least three weeks plus an extra week for the infection to be found and for the immunity to have mounted. Once initiated, the vaccine CTL proliferation was 5.6 fold the natural infection (Pinto et al., 2006), $\gamma = 14$ day^{-1}. When the vaccine does not elicit a cross-reactive response we set $\alpha_{hr} = 0$, otherwise $\alpha_{hr} < \alpha_{16.}$

With the immunity scenarios we asked: Which scenario gave competitive release, and how does the vaccine change the within-host ecology?

Results

Natural Immunity

Figure 2 summarizes the outcome of a wide range of co-infections for different combinations of types that experience no to full cross-reactivity ($0 < \alpha_{16\ or\ hr} < 0.5$) and that experience immunity mounted first against HPV-16 (i) or against the other type (ii). Notice that in all these ecological scenarios coexistence is possible for many combinations of types (see the regions 'all coexist', 'P_{hr} & P_{co}', 'P_{16} & P_{co}' and 'P_{16} & P_{hr}' regions in Fig. 2), demonstrating that coexistence does not solely arise from independence or 'no interactions'.

In the case of independent resource use (Fig. 2a), the outcomes of co-infections with types of various replication rates (from none, $f_{hr} = 0$, to the same replication rate as HPV-16, 0.4), suggest that related types (strong cross-reactivity: $0.25 < \alpha_{16\ or\ hr} < 0.5$) would be pressured to have similar replication rates to HPV-16 in order to avoid clearance (see arrow Fig. 2a.i). However, less cross-reactive types ($\alpha_{hr} < 0.25$) can have lower replication rates ($f_{hr} < 0.4$), and still coexist in co-infections (Fig. 2a.i: 'all coexist' region), regardless of which type is targeted by the immune response. Usually, non-HPV-16 types are found at lower abundances, and to get this discrepancy in viral load under independence requires non-HPV-16 types to have different intrinsic replication rates (Table 2 A; natural, compare *hr* for different f_{hr}) and burst sizes, which, we believe, has not been quantified.

HPV-16 infections tend to last longer than infections with other types (Trottier et al., 2008), yet, it has not been demonstrated that HPV-16 consistently outlasts all other co-infecting types. Reconciling this with our results suggests that strong facilitation is unrealistic because it allows the non-HPV-16 type to dominate all natural infections (Fig. 2 c, d, $\varepsilon > 20$, in both i and ii).

When independent, both types infect the same fraction of patches (see as an example, < 300 days in Fig. 3 a.i in A and B) if colonization rates of patches are medium-high. Clinically, this would lead to finding co-infecting types just as often as HPV-16 by randomly sampling from different regions of the cervix, vagina or vulva, which is not the case. Also, this does not coincide with the clustering of types that is often found within patients (e.g. some types are more common on the vaginal wall or in mucosal cells (Castle et al., 2007; Hadzisejdć et al., 2007)). However, heterogeneous patch use is more characteristic of low colonization rates (not shown) and intra-patch competition (Fig. 3 a.ii in A and B).

Recent data showed a significant decrease in HPV-16 viral load, but not exclusion, when in co-infection with another HR α-9 type (Xi et al., 2009). Here, we compared natural single infection and co-infection viral loads of each type, Table 1 and 2 respectively. A viral load drop of HPV-16 in co-infection, like that found in Xi et al., happened across both immunity conditions (i and ii) and in all competitive and competitive with facilitation scenarios (compare 16 in Table 1 to Table 2 B and D under 'weak'). With facilitation and independence, however, HPV-16's viral load could be higher (compare Table 1 to Table 2 A and C under 'weak'), which is inconsistent with (Xi et al., 2009), thus, competition or competition with facilitation best represented these findings.

Finally, in all natural immunity scenarios, HPV-16 did not exclude the other type before CTL invasion (e.g. see 0 to 300 days in Fig. 3a in A and B) even if replication rates were very different, for example $f_{hr} = 1$ and $f_{16} = 0.01$. This is because the non-HPV-16 type can exist within co-infected patches, unless intra-patch competition is so high that it cannot reproduce at all $f_{hrco} = 0$. This suggests that in all considered interaction scenarios, types can coexist before the adaptive immune system invades to clear the infection. Once again, this shows that independence is not required for coexistence.

Vaccine Conditions

Under vaccine conditions, all non- and most weakly cross-reactive types were not cleared by the vaccine (the large 'P_{hr} wins' regions in Fig. 4 compared to Fig. 2) which corresponds with vaccine trials that showed limited cross-protection (Wheeler et al., 2012). From Figure 4 a – c, it appears that the underlying within-host interactions play little role in determining whether the non-vaccine type is cleared, instead the strength of cross-reactivity best determines whether a

type is cleared ($\alpha_{hr} > 30$). However, if there is both underlying competition and facilitation then the strength of facilitation can allow cross-reactive non-vaccine types to escape (Fig. 4 d). Finally, if non-vaccine types are similar or more reproductive than HPV-16, then through simple competition for empty patches most are able to evade clearance by the vaccine (Fig. 4 e, line).

The non-vaccine type was able to infect newly available patches, instead of residing mostly in co-infected patches (Fig. 3 before 300 days: compare a and b). How quickly the non-vaccine type filled all available patches depended on the underlying interaction and the strength of cross-reactivity (Fig. 3 b in B).

Whether the new patch dominance of the non-cross-reactive non-vaccine type translated to noticeably higher viral loads depended on which natural ecological scenario existed before vaccination. Under independence, weak competition and facilitation only the vaccine loads were lower, though the decrease depended on the strength of the cross-immunity (A, B, C in Table 2). Under strong and moderate competition, the vaccine viral loads were higher (B in Table 2). The degree of competitive release depended on the strength of the intra-patch competition and the strength of the cross-reactivity. For example, with strong competition ($f_{hrco} = 0.02$) non-cross-reactive types doubled their viral load (B in Table 2).

Since our natural immunity results point to some form of competition as being the most likely underlying ecological scenario, then the vaccine could increase viral loads of the non-vaccine types in co-infected vaccine patients, if they are not cross-reactive with the vaccine. Also note that types along the border between clearance and escape (Fig. 4) could experience a strong selection pressure to evolve to become less similar to the vaccine type. The arrows in Figure 4 e show that if the non-vaccine type evolves to be less immunologically distinct or to have a higher replication rate (horizontal and vertical arrows, respectively) then they can escape clearance.

Discussion

The most common hypothesis for HPV type interactions is that they do not interact (Dillner et al., 2010; Stanley et al., 2006; Tota et al., 2013, 2011a; Woodman et al., 2007), and so far, most vaccine trials have not seen significant increases in prevalence of non-vaccine types (Wheeler et al., 2012) which seems to support this independence hypothesis. Similarly, studies using odds ratios have concluded that type replacement is not likely because HPV types were found to occur randomly and to lead to cervical disease independently (Chaturvedi et al., 2011; Palmroth et al., 2012; Rositch et al., 2012; Tota et al., 2013, 2011b). However, two types have been flagged as potentially having a competitive advantage (Merikukka et al., 2011; Tota et al., 2011a) and a recent study found that the prevalence of non-vaccine types, including high-risk types, was indeed higher in vaccinated patients (Kahn et al., 2012). Our study helps reconcile these different findings by illustrating that within-host dynamics can give rise to observed clinical patterns without invoking independence or facilitation. We propose that we can help explain surprising results if we adopt an ecological perspective and if we are mindful of which scale we are studying.

Consider three scales: the tissue (or patch) level, individual hosts, and the host population. At the tissue level, there are some suggestions of type interactions. HPV-40 and HPV-11 can separate regionally (Christensen et al., 1997), and recently a study showed that lesions are caused by only one HPV type (Quint et al., 2012). These results could be explained by spatial ecology concepts such as ‘local founder control’ (the first in a patch blocks the other from entering) or ‘hierarchical competition’ (the superior local competitor always, quickly or slowly, outcompetes the other) (Klausmeier and Tilman, 2002), and thus, should be considered.

Indeed, HPV's highly spatial infection cycle suggests that more complex interactions are at work than is appreciated. Common clinical methods (e.g. swabs) are too coarse to see these differences in HPV tissue distribution (Mendez et al., 2005; Rousseau et al., 2003) and thus are unlikely to find differential uses of space by HPV types. Therefore, there is considerable need for novel animal model studies or other experimental approaches to address HPV type interactions at the tissue level.

At the host level, HPV types have consistently been found to co-infect (Plummer et al., 2011). Here, we have shown that various patch-level interactions can lead to coexistence at the host level. Hence, coexistence of types inside a patient does not necessarily imply that types are not interacting. This is consistent with ecological findings where competitive interactions regularly do not lead to exclusion. In the HPV literature, competitive exclusion is considered the ultimate outcome of 'niche overlap' (Garnett and Waddell, 2000; Poolman et al., 2008; Tota et al., 2013), however, ecological niche theory (Chase and Leibold, 2003; Tilman, 1982) is more developed than this and gives several mechanisms for coexistence. Consider four key dimensions of a niche: resources, enemies, space, and time (Chesson, 2000). Species (or strains) will differ in more than one of these, and trade-offs play an important role in mediating their coexistence (Amarasekare, 2009; Chesson, 2000). For example, stable coexistence between strains can be due to a trade-off where the advantage of immunity evasion by one strain is balanced by the advantage of resource exploitation of the other. Because antigenically similar strains that exploit the same host are very common, this trade-off should be investigated more in HPV and in other virus studies (Murall et al., 2012). Similarly, differences in the use of space readily allow for coexistence (review (Amarasekare, 2003)). Our model shows how this may occur in HPV, because patch heterogeneity allowed types to coexist at the host level. Finally, then, our results and present day understanding of ecological coexistence suggest that the assumption that the

presence of co-infections is evidence of no interactions between types (Kaasila et al., 2009; Palmroth et al., 2012) is fallacious.

Returning to scale, inferring interactions at the population level for HPV, and other infectious diseases with regularly co-infecting strains, may not be possible given the aforementioned. Indeed, it is proving a difficult task (review (Tota et al., 2013)). Since species distributions do not always hint at the underlying local interactions, more direct empirical studies at lower scales are needed, and finding competitive interactions will require manipulative experiments. Untangling these interactions is very important because the response to a perturbation, such as vaccination, will vary widely depending on the underlying mechanism, i.e. how types interact.

It should be noted that 'no interactions', or as Hubbell put it "nothing is going on", is not equivalent to conventional ecological 'neutrality', where types would still be interacting but their effect on each other would be exactly symmetrical and thus their per capita vital rates would not differ (Hubbell, 2001). A within-host model that captures neutrality would be inspired by a neutral model such as Hubbell (2001), which has been attempted for a multi-strain epidemiological model (Lipsitch et al., 2009). Inadvertently, the issue of HPV type interactions has landed in the centre of an ecological debate on whether niche or neutral theory best describes ecological interactions (Bell, 2001; Chase and Myers, 2011; Gravel et al., 2006; Hubbell, 2001; Rosindell et al., 2012). Its resolution should, therefore, be of interest to both the medical and ecological communities. Since closer examination of ecological systems often uncovers underlying non-neutral interactions (Holt, 2006; McGill et al., 2006), and since many medically important viruses are highly competitive (e.g. HIV), conclusively finding independent niches or neutrality in HPV would be very interesting indeed.

Since our model suggests that some competition is likely, non-vaccine types in vaccinated hosts could increase patch use and viral load. To observe this signal, clinical studies would be required, e.g. ones that compare the viral loads (preferably longitudinal) of non-vaccine types in vaccinated and unvaccinated hosts, in order to capture any niche expansion (or contraction). Our model shows that niche expansion could be slight and so we suspect this within-host signature will be detectable before there are significant changes in prevalence at the population level.

Polyvalent vaccines are currently being developed against HPV and there is discussion as to which types to include (Bosch et al., 2008). Inclusion of all the oncogenic types in the polyvalent vaccines would be more effective than depending on weak cross-protection. Our model shows that vaccines must elicit a strong cross-reactive response in order to be effective against non-vaccine types. One promising approach to achieve this maybe to include the L2 protein which appears to elicit a broader cross-neutralizing effect (Mariani and Venuti, 2010).

There are some limitations and caveats to consider. First, this model is not explicitly spatial. However, because HPV interact either within or between patches, and disperse to either close or far new patches, then this kind of setup is most appropriately modeled by patch models (Klausmeier and Tilman, 2002). Second, we did not include a dynamical model of antibodies. Our model assumes that the antibody response is constant and so, does not capture the more realistic feature of the delay before invasion of the infection area. Thus, our model may be over estimating the effect of the vaccine since a delay would benefit the virus to establish a secondary infection. Whether this constant assumption or a dynamical approach is needed will depend on the empirical measurement of the dynamics of the humoral response directly after a secondary infection. Finally, while we assumed that co-infected cells experience additive clearance, it is possible that by presenting the antigens of two types, presentation and thus clearance are

decreased. If this were true, then it would be advantageous for the viruses to co-infect cells, particularly when in a co-infection with immunologically distinct types. This could result in more co-infected patches, and potentially longer transients before clearance, as it would be more difficult to clear the targeted type as it hides in co-infected patches that act as reservoirs.

Despite these caveats, mathematical models are often used to help untangle complex interactions in ecology and in disease dynamics (Mideo et al., 2008), and are particularly useful when there are knowledge gaps. Although our model is a simplification of reality, it is grounded in the biology of HPV infections, and so it allowed us to explore the competing hypotheses from the literature.

Though we do not directly address the evolutionary potential of HPV, our results suggest that vaccinated hosts could set the stage for some non-vaccine cross-reactive types to escape the vaccine response. Vaccine trials have demonstrated that cross-protection is partially effective (e.g. 44.8% effective against 6-month persistent HPV-33 infection (Wheeler et al., 2012)), and so this 'leakiness' might select for variants to escape this new vaccine-induced immune response. Our results point to two traits that could allow variants of non-vaccine types to escape: decreased epitope similarity, or more alarmingly, increased replication rates. In time, then, vaccine cross-protection could wane, i.e. *'cross-immunity escape'*, or non-vaccine types could become more aggressive. Unfortunately, vaccines have driven other pathogens to increase their replication rates (Gandon and Day, 2008). Rapid ecological changes drive evolutionary changes and not enough is known about the differences between the natural and vaccine within-host ecology to be certain that the vaccine will not select for trait changes in non-vaccine types.

Currently, our model does not lend itself to model fitting since enumerating infected patches from samples is not yet implemented. However, with new methods (e.g. (Quint et al.,

2012)) it could be possible, and we hope future work will link models with patch data. As we move to using more ultrasensitive HPV genotyping assays (Schmitt et al., 2010), we can more frequently sample co-infections, in order to better quantify the dynamics of all types in the infection. This will help to measure natural *vs.* vaccine within-host differences; fit models to data and hence better tease apart the within-host ecology; see if non-vaccine types in co-infections are reciprocally affected by the vaccine types; and, finally, get direct evidence for the duration over which vaccinated hosts produce and shed virus relative to unvaccinated hosts. Instead of the 'wait and see' approach of long-term monitoring of type prevalence in vaccinated populations, we hope this work will incite new proactive studies.

More ecologically cognisant HPV studies will help explain whether the vaccine drives or avoids an evolutionary ecological response. They will either lead us to remedy the problem of type replacement if it appears, or help us understand more mechanistically why the vaccine worked. This knowledge can then help us avoid type replacement in future vaccination programs.

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Figure Legends

Figure 1.

a) An illustration of layered squamous cell infections; a top-down and cross-section view of the epidermis. Free virus particles are released at the surface (i) and need an abrasion in the epidermis to reach basal cells to start a new patch (ii). **b)** A schematic of the model. See methods for description of symbols.

Figure 2.

Parameter plots: infection outcomes for various ecological scenarios. a) Independence. At $f_{hr} = 0.4$ (dotted line), the two types reproduce at the same rate. In the bottom-left quarter of these two plots (below $f_{hr} = 0.4$ and below $\alpha_{16\ or\ hr} = 0.25$) the non-vaccine type has a lower reproductive rate than HPV-16 and the types are weakly cross-reactive. **b) Intra-patch competition. i.** When cross-reactivity is weak ($\alpha_{hr} < 0.25$) then the strength of the intra-patch competition affects the outcome. At $f_{hrco} = 0$, the intra-patch competition is so strong that the other type is excluded from patches altogether. **ii.** Only when intra-patch competition is weak, is the HR type able to evade the immune response by hiding in co-infected patches (P_{16} & P_{co} region). **c) Facilitation.** Facilitation allows for large regions of coexistence, even with a small amount of facilitation ($1 < \varepsilon < 12$). **d) Facilitation and competition.** The inclusion of intra-patch competition shrinks the coexistence region, and competition decreases the ability of facilitation to release the other type from clearance by the immune system.

Figure 3.

Example time-series of natural and vaccine cases (neutral, competition, facilitation).

A. Types are not cross-reactive. **B.** Types are weakly cross-reactive. **a) Natural cases.** In all natural immunity scenarios with interactions (ii and iii) both types coexist until the immune invasion (before day 300), i.e. co-infecting patches prevents exclusion of less replicative type. Intra-patch competition (ii) gives more heterogeneous patch use and HPV-16 dominance, whereas co-infected patches dominate in facilitation case (iii) which is unrealistic. **b)** Under vaccination, non-vaccine types are able to infect all patches once the vaccine type is cleared, in A faster than in B.

Figure 4.

Parameter plots of vaccine conditions: outcomes for various ecological scenarios.

a) Independence. For non- and weakly cross-reactivity types the vaccine allows for the non-vaccine type to infect all patches, regardless of its replication rate. **b) Intra-patch competition.** As intra-patch competition increases the clearance region grows slightly. **c) Facilitation.** The vaccine results are independent of facilitation; similar to independence, where strength of cross-reactivity determines outcome. **d) Facilitation and Competition.** Combining facilitation and competition shrinks clearance region, allowing the non-vaccine type to escape. **e) Patch competition.** Used model A.2. Compared to the neutral parameter plot (a) the clearance region is more affected by competition for patches. Increasing the replication rate of the non-vaccine type is more effective at allowing the type to avoid clearance by the vaccine even if it experiences strong cross-reactivity (arrow up). Types that are more cross-reactive could evolve to increase its replication rate

to similar or even higher replication rates than HPV-16 to avoid being wiped out by the vaccine.

Table 1.

Mean viral loads: single infection. Mean quantities of free virions produced by patches infected by each HPV type when in an infection alone. Made with model A.1. *Parameters*: $f_{16} = 0.4$, f_{hr} = see table, $\alpha_{16\ or\ hr} = 0.5$, $e_{16\ or\ hr}=1$, $w_{16\ or\ hr} = 1$, $\mu = 0.5$, $\gamma = 2.5$.

Table 2.

Mean viral loads: co-infection. Mean quantities of free virions produced by all patches that contain a particular type per day. These are not meant to represent real measurements of viral titers but rather are simply a method of measuring the relative viral loads of various hosts. *Parameters*: see Table A.2 (Appendix).

Appendix A

Patch model derivation

Assume that the first patch of infected cells is established during, or shortly thereafter, physical contact (e.g. intercourse) with an infected part of another person's body. Given that there are a number of abrasions in the epithelium, there are now two kinds of patches, a portion of which are empty and some that are infected with the HPV type HPV-16, P_{16}. The proportion of empty patches is thus $P_0 = 1 - P_{16}$. We assume that the proportion of infected patches changes dynamically in time, and their creation is due to the infection of empty patches at a rate, e_{16}, by the free virions produced by the infected patch, f_{16}. Assuming mass-action and using a similar formalism as the Levins' model (Klausmeier and Tilman, 2002; Levins and Culver, 1971; Levins, 1969) we get the equation $\frac{dP_{16}}{dt} = e_{16} f_{16} P_{16} P_0$ for the creation of infected patches.

Since clearance of HPV infection requires CTL invasion (Stanley, 2006), patches are only cleared by CTL. A variable that represents the CTL population, *Z*, is included because CTL invasion is a dynamic process. Therefore, a singly infected patch is cleared by the immune system at a rate α_{16}. We assume that the abundance of CTL increases proportionally with the infected patches. Also to investigate how antibodies decrease the establishment rate of new patches by neutralizing free virions, we included the parameter w_{16}. All together this gives a patch model of an infection with only HPV-16,

$$\begin{aligned}
\frac{dZ}{dt} &= \gamma P_{16} Z - \mu Z \\
\frac{dP_{16}}{dt} &= \frac{e_{16}}{w_{16}} f_{16} P_{16} P_0 - \alpha_{16} P_{16} Z \\
P_0 &= 1 - P_{16}
\end{aligned} \tag{A.1}$$

Consider now a co-infection case, where two HPV types solely infect their own patches. The immune response is only stimulated by one type, *i*, but can kill the other type, *j*, if there is cross-reactivity (where $\alpha_j > 0$). Thus, model A.1 can be extended then to,

$$\begin{aligned}
\frac{dZ}{dt} &= \gamma P_i Z - \mu Z \\
\frac{dP_{16}}{dt} &= \frac{e_{16}}{w_{16}} f_{16} P_{16} P_0 - \alpha_{16} P_{16} Z \\
\frac{dP_{hr}}{dt} &= \frac{e_{hr}}{w_{hr}} f_{hr} P_{hr} P_0 - \alpha_{hr} P_{hr} Z \\
P_0 &= 1 - P_{16} - P_{hr}
\end{aligned} \tag{A.2}$$

This model assumes that HPV-16 and the other HR type are competing for empty patches, and cannot co-infect patches. Note that this model could be altered to represent a within-host superinfection scenario. P_{16} could become P_{hr} and *vice versa*, by allowing infected patches to become infected by the other type and by allowing instantaneous replacement of the resident type. We chose not to consider this scenario in our analyses.

To consider independence, we included two empty patch variables,

$$\begin{aligned}
\frac{dZ}{dt} &= \gamma P_{16} Z - \mu Z \\
\frac{dP_{16}}{dt} &= \frac{e_{16}}{w_{16}} f_{16} P_{16} P_{016} - \alpha_{16} P_{16} Z \\
\frac{dP_{hr}}{dt} &= \frac{e_{hr}}{w_{hr}} f_{hr} P_{hr} P_{0hr} - \alpha_{hr} P_{hr} Z \\
P_{016} &= 1 - P_{16} \\
P_{0hr} &= 1 - P_{hr}
\end{aligned} \tag{A.3}$$

Here the two HPV types do not use the same patches, and are completely independent in their resource use. If the HPV types are also immunologically distinct, then this model, A.3, represents an 'independent niche' scenario, which is not consistent with the conventional use of the word 'neutral' in the ecology literature (see discussion for more).

In order to investigate the scenario where patches could be co-infected by both HPV types we included the variable, P_{co}. This implies that free virions of type i (either *16* or *hr*) are now produced by both co-infected patches, $P_{co,}$ and singly infected patches, $P_{i,}$ at a rate of f_{ico} and f_i respectively. Thus, the terms that represent viral production, $f_i P_i$, are now replaced by the additive term ($f_i P_i + f_{ico} P_{co}$).

$$\begin{aligned}
\frac{dZ}{dt} &= \gamma (P_i + P_{co}) Z - \mu Z \\
\frac{dP_{16}}{dt} &= \frac{e_{16}}{w_{16}} (f_{16} P_{16} + f_{16co} P_{co}) P_0 - \frac{e_{hr16}}{w_{hr}} (f_{hr} P_{hr} + f_{hrco} P_{co}) P_{16} - \alpha_{16} P_{16} Z \\
\frac{dP_{hr}}{dt} &= \frac{e_{hr}}{w_{hr}} (f_{hr} P_{hr} + f_{hrco} P_{co}) P_0 - \frac{e_{16hr}}{w_{16}} (f_{16} P_{16} + f_{16co} P_{co}) P_{hr} - \alpha_{hr} P_{hr} Z \\
\frac{dP_{co}}{dt} &= \frac{e_{16hr}}{w_{16}} (f_{16} P_{16} + f_{16co} P_{co}) P_{hr} + \frac{e_{hr16}}{w_{hr}} (f_{hr} P_{hr} + f_{hrco} P_{co}) P_{16} - (\alpha_{16} + \alpha_{hr}) P_{co} Z \\
P_0 &= 1 - P_{16} - P_{hr} - P_{co}
\end{aligned} \tag{A.4}$$

where i is either *16* or *hr* depending on which type is targeted by the immune response. We assumed that co-infected patches cannot be created by a single instantaneous event by both HPV-16 and the other HR type (because it is fairly unlikely that one cell in an empty patch be infected

by two different types at exactly the same time), i.e. P_0 cannot be come P_{co} without first being P_{16} or P_{hr}. Therefore, P_{co} grows only by these two two terms. Also two new establishment rates, e_{ij}, are needed to capture the establishment of type i into a patch already infected with type j. Also, we assumed that co-infected patches cannot become singly infected because a patch can contain co-infected cells, and thus CTL cannot remove a single type from co-infected patches.

Co-infected patches are also removed by CTL, and at the same rates as single infected patches, a_i or a_j. However, since co-infected cells will present antigen from both types, co-infected patches are assumed to be more visible to the CTL response if the immune response is cross-reactive, thus the additive term $\alpha_{16} + \alpha_{hr}$ in the co-infected patch equation.

To simplify the model, both types are set to have the same establishment rates, $e_{16} = e_{hr} = e$. Also, we assumed that the positive effect of the presence of one type on the establishment rate of the second type is unidirectional, i.e. only the presence of HPV-16 benefits the entry of other non-HPV-16 types not vice versa, thus $e_{16} = e_{16hr} = e$ and $e_{hr} \neq e_{hr16} = \varepsilon$.. The biological mechanism for facilitation is not clear yet (Elbasha and Galvani, 2005) but it is suggested that infection of HPV-16 may facilitate infection by another type (Mendez et al., 2005). This stems from epidemiological findings of very frequent sequential and concurrent infections where by HPV-16 is more common (Rousseau et al., 2001) and is thus potentially facilitating infections by other less common types. Therefore, we consider facilitation (positive effect in one direction) and not mutualism (reciprocal positive effect). Note then that because the HR type more readily enters patches already infected with HPV-16 this implies $\varepsilon > e$.

Together, this gives the final version of the model used for analysis of different interaction scenarios (except independence) under natural immunity,

$$\frac{dZ}{dt} = \gamma(P_i + P_{co})Z - \mu Z$$
$$\frac{dP_{16}}{dt} = \frac{e}{w_{16}}(f_{16}P_{16} + f_{16co}P_{co})P_0 - \frac{\varepsilon}{w_{hr}}(f_{hr}P_{hr} + f_{hrco}P_{co})P_{16} - \alpha_{16}P_{16}Z$$
$$\frac{dP_{hr}}{dt} = \frac{e}{w_{hr}}(f_{hr}P_{hr} + f_{hrco}P_{co})P_0 - \frac{e}{w_{16}}(f_{16}P_{16} + f_{16co}P_{co})P_{hr} - \alpha_{hr}P_{hr}Z \quad \text{(A.5)}$$
$$\frac{dP_{co}}{dt} = \frac{e}{w_{16}}(f_{16}P_{16} + f_{16co}P_{co})P_{hr} + \frac{\varepsilon}{w_{hr}}(f_{hr}P_{hr} + f_{hrco}P_{co})P_{16} - (\alpha_{16} + \alpha_{hr})P_{co}Z$$
$$P_0 = 1 - P_{16} - P_{hr} - P_{co}$$

This is model 1 in the methods section.

Note that the terms ($f_i P_i + f_{ico} P_{co}$) are measures of viral load in these scenarios, because ($f_i P_i + f_{ico} P_{co}$) is the total number of type i virions produced by patches P_i and P_{ij} at one time step. We used these terms to calculate viral loads for the various ecological scenarios.

Parameter considerations

When comparing vaccination to natural immunity, we assumed the vaccine's cross-reactivity scaled linearly in strength, e.g. if the natural cross-reactive attack rate against the non-vaccine type is $\alpha_2 = 0.1$, and if the vaccine is 100 times stronger, then the vaccine cross-reactive attack rate is $\alpha_2 = 10$. This then assumes that non-vaccine types in vaccinated hosts experience stronger cross-reactive immune response, and therefore, we considered the region $0 < \alpha_2 < 50$ in the vaccine parameter plots. If we had not made this assumption then we would consider a very small region of the vaccine plots ($\alpha_2 < 0.5$).

Initial conditions were chosen to be as realistic as possible to the initiation of an infection. Initial P_{16} and P_{hr} were set to be the same and small ($P_{16}(0) = P_{hr}(0) = 0.01$) to represent the initial colonization of patches (without giving either strain an advantage). P_{co} was

set to zero because it is unlikely they immediately infect the same patch. Finally at the time of immunity invasion, one CTL invades ($Z(0) = 1$). We considered how changing some of these initial conditions could change results. First we found that increasing initial Z implied that CTL would invade much sooner, which is unrealistic for HPV infections. Also, higher initial Z also lead to vaccine results where almost all non-vaccine types (even weakly cross-reactive) would be cleared by the vaccine. This is also unrealistic since vaccine trials show that only some related types are cross-protected against.

Table A.1: Parameter restrictions and ranges (for types *i* and *j*)

Strain parameters		
e and ε	establishment rate	When e =1, then every time a virion encounters a patch it establishes successfully. We assume facilitation when $\varepsilon > e$ and when entry into P_{I6} is easier than into P_0 for the HR type, thus $\varepsilon > 1$. There is no reason to believe that HPV-16 blocks entry of the other type (i.e. ε cannot be less than e). Overall then $\varepsilon \geq 1$.
f_i and f_{ij}	rates of virion production by strains inside patches alone and together	If $f_i = 1$, then one virion is made per patch/unit time. If $f_i > 1$, then more than one virion is made per patch/unit time. And, if $0 \leq f_i < 1$, then less than one virion is made per patch/unit time. Therefore, virion production rates should be $f_i \geq 0$ and $f_{ij} \geq 0$. However, given that there are many cells per patch, at least one cell will burst at any given time, this implies that $f_i > 1$ and $f_{ij} > 1$.
Immunity parameters*		
α_i	CTL killing rate	If $\alpha_i = 1$, then CTL killing is 100% successful, i.e. each CTL removes one patch per unit time. If $0 \leq \alpha_i \leq 1$, then one CTL removes less than one patch per unit time. If $\alpha_i > 1$, then each CTL removes >1 patch per unit time. We assume CTL are not completely efficient, i.e. $0 \leq \alpha_i \leq 1$, in the natural immunity cases. [Note: this does not mean that one CTL does not kill >1 patch in its lifespan it just does not necessarily kill 1 patch at every single time step.]
w_i	strength of neutralizing antibodies	When $w_i = 1$ there is no antibody response. When $w_i > 1$, then w_i decreases the rate of establishment.

Table A.2: Parameters used for figures and tables

	Parameters							Model used
	in all subplots	**in specific subplots**						
Figure 2	$f_{16} = 0.4$ $\gamma = 2.5$ $\mu = 0.5$ $w_{16} = 1$ $w_{hr} = 1$ $e = 1$	**(i)** $\alpha_{16} = 0.5$ α_{hr} varies from 0 to 0.5		**(a)**	f_{hr} varies from 0 to 0.8 $e_{16} = e_{hr} = 1$			A.3
				(b)	$f_{hr} = 0.2$ $f_{16co} = 0.2$ f_{hrco} varies from 0 to 0.2 $\varepsilon = 1$			A.5
		(ii) $\alpha_{hr} = 0.5$ α_{16} varies from 0 to 0.5		**(c)**	$f_{hr} = 0.2$ $f_{16co} = 0.4$ $f_{hrco} = 0.2$ ε varies from 1 to 51			A.5
				(d)	$f_{hr} = 0.2$ $f_{16co} = 0.2$ $f_{hrco} = 0.05$ ε varies from 1 to 51			A.5
Figure 3	$f_{16} = 0.4$ $f_{hr} = 0.2$ $\mu = 0.5$ $e = 1$	**A**	**(a)** natural: $\gamma = 2.5$ $\alpha_{16} = 0.5$ $\alpha_{hr} = 0$ $w_{16} = w_{hr} = 1$	**(b)** vaccine: $\gamma = 14$ $\alpha_{16} = 50$ $\alpha_{hr} = 0$ $w_{16} = 100$ $w_{hr} = 1$	**(i)**	$e_{16} = e_{hr} = 1$		A.3
					(ii)	$f_{16co} = 0.2$ $f_{hrco} = 0.05$ $\varepsilon = 1$		A.5
		B	*Same as in A except:* **(a)** natural: $\alpha_{hr} = 0.05$	**(b)** vaccine: $\alpha_{hr} = 5$ $w_{hr} = 10$	**(iii)**	$f_{16co} = 0.4$ $f_{hrco} = 0.2$ $\varepsilon = 34$		A.5
Figure 4	$f_{16} = 0.4$ $\alpha_{16} = 50$ α_{hr} varies from 0 to 50 $\gamma = 14$ $\mu = 0.5$ $w_{16} = 100$ $w_{hr} = 2(\alpha_{hr})$ $e = 1$	**(a)**	f_{hr} varies from 0 to 0.8 $e_{16} = e_{hr} = 1$					A.3
		(b)	$f_{hr} = 0.2$ $f_{16co} = 0.2$ and f_{hrco} varies from 0 to 0.2 $\varepsilon = 1$					A.5
		(c)	$f_{hr} = 0.2$ $f_{16co} = 0.4$ and $f_{hrco} = 0.2$ ε varies from 1 to 51					A.5
		(d)	$f_{hr} = 0.2$ $f_{16co} = 0.2$ and $f_{hrco} = 0.05$ ε varies from 1 to 51					A.5
		(e)	f_{hr} varies from 0 to 0.8 $e_{16} = e_{hr} = 1$					A.2
Table 2.	$f_{16} = 0.4$ $\mu = 0.5$ $e = 1$	**Natural** $\gamma = 2.5$, $w_{16\ and\ hr} = 1$ **none:** *if* $\alpha_i = 0.5$, then $\alpha_j = 0$ **weak:** *if* $\alpha_i = 0.5$, then $\alpha_j = 0.2$ **Vaccine** $\gamma = 14$, $\alpha_{16} = 50$, $w_{16} = 100$ **none:** $\alpha_{hr} = 0$ $w_{hr} = 1$ **weak:** $\alpha_{hr} = 20$ $w_{hr} = 40$		**A**	f_{hr} see table $e_{16} = e_{hr} = 1$			A.3
				B	$f_{hr} = 0.2$ f_{hrco} see table $f_{16co} = 0.2$ $\varepsilon = 1$			A.5
				C, D	$f_{hr} = 0.2$ $f_{16co} = 0.4$ in C and = 0.2 in D $f_{hrco} = 0.2$ in C and = 0.05 in D ε see table			A.5

Table 1.

		Natural	
		against 16	against HR
16		190.3	-
hr	f_{hr}		
	0.35	-	166.2
	0.2	-	94.0
	0.05	-	21.8

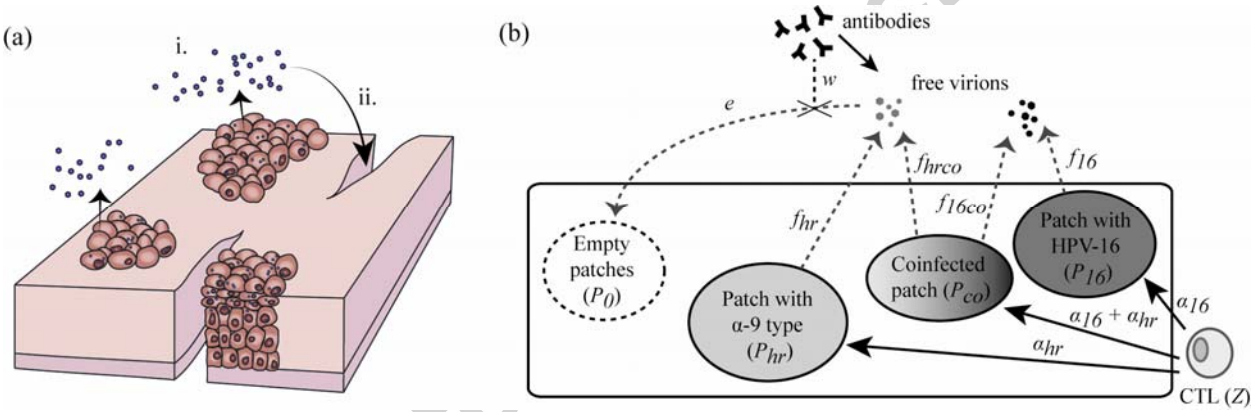
Table 2.

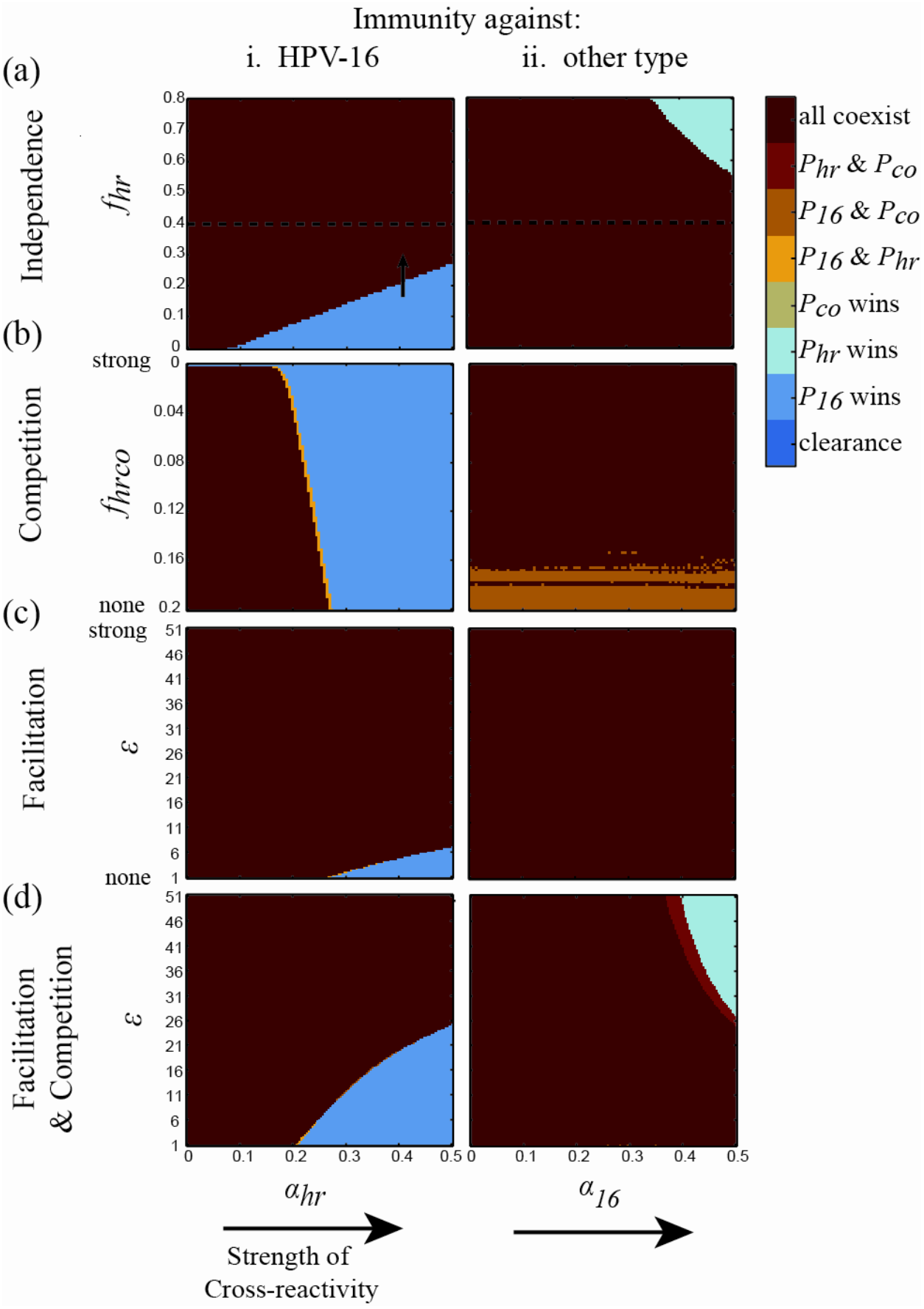
				Natural				Vaccine	
				i. against 16		ii. against HR			
		cross-reactivity:		none	weak	none	weak	none	weak
A		f_{hr}							
Neutrality		0.35	16	186.7	187.7	234.6	230.4	2.3	2.3
			hr	204.8	199.9	162.8	162.8	112.5	12.4
		0.2	16	186.7	186.7	234.6	232.1	2.3	2.3
			hr	115.1	110.3	91.0	91.0	63.4	6.2
		0.05	16	186.7	186.7	234.6	233.8	2.3	2.3
			hr	25.6	21.5	19.3	19.3	14.5	0.6
B		f_{hrco}							
Intra-patch	strong	0.02	16	109.0	120.5	165.5	164.3	1.3	1.9
Competition			hr	32.2	7.1	7.1	7.1	61.0	6.7

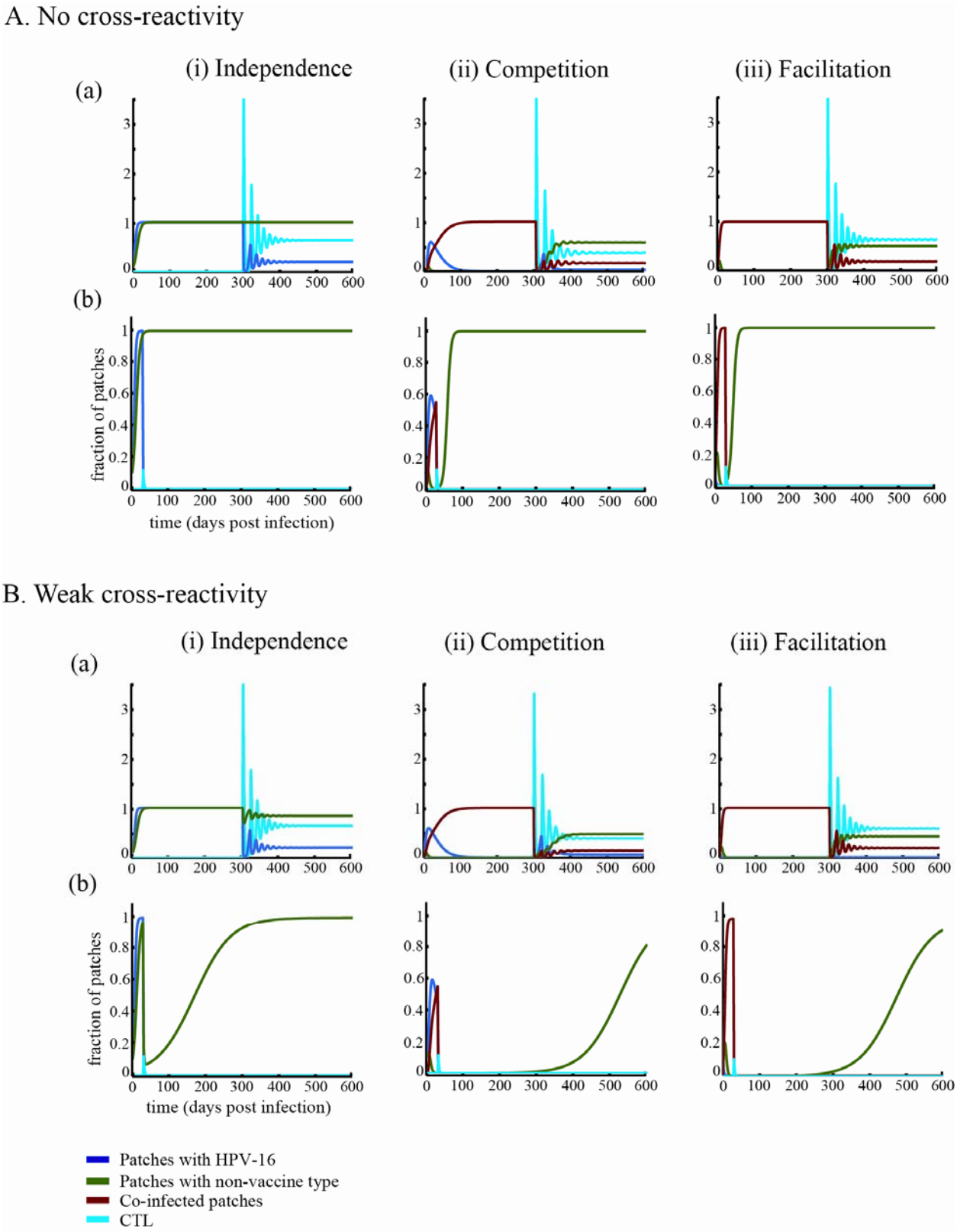
	moderate	0.1	16	92.3	130.7	149.2	147.8	1.3	1.9
			hr	68.3	42.2	41.8	41.8	61.2	6.7
	weak	0.18	16	90.2	101.2	147.3	145.8	1.3	1.9
			hr	103.7	78.7	76.6	76.6	61.5	6.7
C		ε							
Facilitation	moderate	34	16	175.2	175.2	231.7	230.5	1.8	1.9
			hr	115.5	114.0	88.3	88.3	62.5	8.1
	weak	6	16	175.2	186.7	232.7	231.2	1.8	1.9
			hr	115.1	97.4	87.8	87.7	62.1	6.9
D		ε							
Facilitation	moderate	34	16	86.1	86.0	142.1	140.9	0.9	1.7
(with			hr	48.8	47.4	22.8	22.8	61.3	7.8
Competition)									
	weak	6	16	88.0	99.0	144.2	143.0	1.0	1.8
			hr	48.2	22.8	22.3	22.2	61.2	6.8

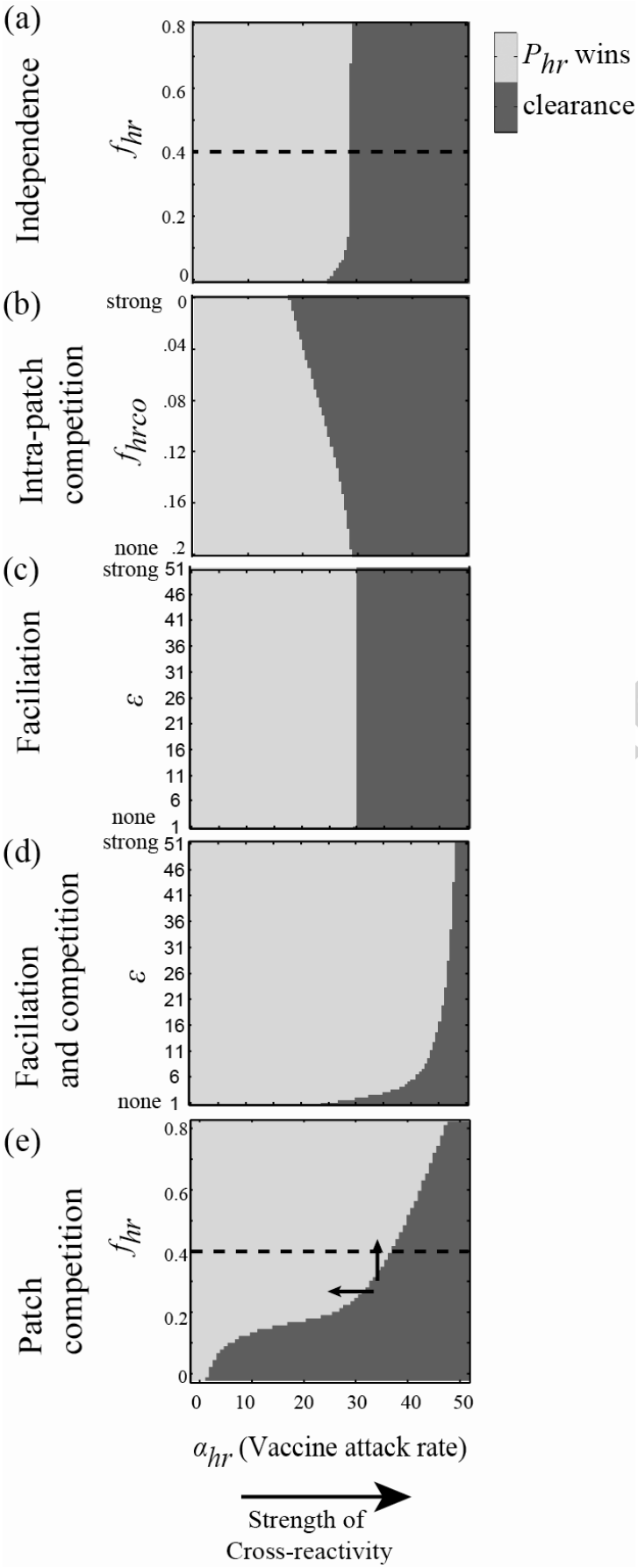
Highlights

- With a model we evaluated competing hypotheses of HPV type interactions
- Independence and facilitation are not necessary for coexistence of types inside hosts
- Spatial heterogeneity can lead to underappreciated complex type-type interactions
- Conditions for type replacement are possible at the within-host level




Immunity against:
i. HPV-16
ii. other type
(a)
Independence
f_{hr}
(b)
Competition
f_{hrco}
strong
none
(c)
Facilitation
ε
strong
none
(d)
Facilitation & Competition
ε
all coexist
P_{hr} & P_{co}
P_{16} & P_{co}
P_{16} & P_{hr}
P_{co} wins
P_{hr} wins
P_{16} wins
clearance
α_{hr}
α_{16}
Strength of Cross-reactivity


A. No cross-reactivity
(i) Independence
(ii) Competition
(iii) Facilitation
(a)
(b)
fraction of patches
time (days post infection)
B. Weak cross-reactivity
(i) Independence
(ii) Competition
(iii) Facilitation
(a)
(b)
fraction of patches
time (days post infection)
Patches with HPV-16
Patches with non-vaccine type
Co-infected patches
CTL


(a)
Independence
f_{hr}
0.8
0.6
0.4
0.2
0
P_{hr} wins
clearance
(b)
Intra-patch competition
f_{hrco}
strong
0
.04
.08
.12
.16
.2
none
(c)
Faciliation
ε
strong
51
46
41
36
31
26
21
16
11
6
1
none
(d)
Faciliation and competition
ε
strong
51
46
41
36
31
26
21
16
11
6
1
none
(e)
Patch competition
f_{hr}
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
α_{hr} (Vaccine attack rate)
Strength of
Cross-reactivity