

Supporting information

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SI Text A

Extension to n Modules: Equivalent Modules. Here we develop the theory for the general case of n equivalent modules. The case of nonequivalent modules is treated in *SI Text B*. Thus, consider an organism consisting of n equivalent modules. Performance is then a function of an n -dimensional trait vector $\theta = (\theta_1, \dots, \theta_n)$. Approximating $F_i(\theta)$ by a second-order Taylor polynomial around the maximum in the constrained trait space $\theta^* = (\theta^*, \dots, \theta^*)$ gives

$$\begin{aligned} F_i(\theta) &\approx F_i(\theta^*) + D(F_i)(\theta^* - \theta) + \frac{1}{2}(\theta^* - \theta)H(F_i)(\theta^* - \theta)^T \\ &\approx F_i(\theta^*) + \sum_{k=1}^n \frac{\partial F_i(\theta)}{\partial \theta_k} (\theta^* - \theta_k) + \frac{1}{2} \sum_{k=1}^n \frac{\partial^2 F_i(\theta)}{\partial \theta_k^2} (\theta^* - \theta_k)^2 \quad [\text{S1}] \\ &\quad + \sum_{\substack{k,l \in \{1, \dots, n\} \\ k \neq l}} \frac{\partial^2 F_i(\theta)}{\partial \theta_k \partial \theta_l} (\theta^* - \theta_k)(\theta^* - \theta_l), \end{aligned}$$

where $D(F_i)$ denotes the gradient of $F_i(\theta)$ and $H(F_i)$ denotes the Hessian matrix of $F_i(\theta)$. All derivatives in *SI Text A* are evaluated at the point $\theta = \theta^*$. Then, for equivalent modules

$$\frac{\partial F_i(\theta)}{\partial \theta_k} = \frac{\partial F_i(\theta)}{\partial \theta_l}, \quad \frac{\partial^2 F_i(\theta)}{\partial \theta_k^2} = \frac{\partial^2 F_i(\theta)}{\partial \theta_l^2}, \quad \frac{\partial^2 F_i(\theta)}{\partial \theta_k \partial \theta_l} = \frac{\partial^2 F_i(\theta)}{\partial \theta_m \partial \theta_n}$$

for all $k, l, m, n \in \{1, \dots, n\}$ with $k \neq l$ and $m \neq n$. These equalities allow us to introduce the following short-hand notation:

$$\begin{aligned} \alpha_i &:= F_i(\theta^*) \\ \beta_i &:= \frac{\partial F_i(\theta)}{\partial \theta_k} \text{ for all } k \in \{1, \dots, n\} \\ \gamma_i &:= \frac{\partial^2 F_i(\theta)}{\partial \theta_k^2} \text{ for all } k \in \{1, \dots, n\} \\ \delta_i &:= \frac{\partial^2 F_i(\theta)}{\partial \theta_k \partial \theta_l} \text{ for all } k, l \in \{1, \dots, n\} \text{ with } k \neq l. \end{aligned}$$

If the phenotypes of the modules fall into two discrete groups, characterized by θ_1 and θ_2 , then the n -dimensional trait space can be reduced to a 2D trait space. We denote performance of an organism with pn modules characterized by θ_1 and $(1-p)n$ modules characterized by θ_2 for the i th task by $F_{i,p}(\theta_1, \theta_2)$. Here $p \in [0, 1]$ is such that $pn, (1-p)n \in \mathbb{N}$. The approximation Eq. S1 can now be rewritten as

$$\begin{aligned} F_{i,p}(\theta_1, \theta_2) &\approx \alpha_i + pn\beta_i(\theta^* - \theta_1) + (1-p)n\beta_i(\theta^* - \theta_2) \\ &\quad + \frac{1}{2}(pn\gamma_i(\theta^* - \theta_1)^2 + (1-p)n\gamma_i(\theta^* - \theta_2)^2) \\ &\quad + pn(pn-1)\delta_i(\theta^* - \theta_1)^2 + (1-p)n((1-p)n-1)\delta_i(\theta^* - \theta_2)^2 \\ &\quad + 2p(1-p)n^2\delta_i(\theta^* - \theta_1)(\theta^* - \theta_2). \end{aligned} \quad [\text{S2}]$$

Let us denote the fitness function of an organism with n modules, pn of which are characterized by θ_1 and $(1-p)n$ of which are characterized by θ_2 , with $\rho_{n,p}(F_{1,p}, F_{2,p})$.

In the case of equivalent modules the point (θ^*, θ^*) is an extremum of the fitness landscape. Its properties are determined by the Hessian matrix of the fitness function evaluated at (θ^*, θ^*) . The Hessian matrix $H = [h_{ij}]$ has entries

$$\begin{aligned} h_{11} &= np(x + py) \\ h_{22} &= n(1-p)(x + (1-p)y) \\ h_{12} &= h_{21} = n(1-p)py, \end{aligned}$$

where

$$x = c(\beta_1(\gamma_2 - \delta_2) - \beta_2(\gamma_1 - \delta_1)), \quad [\text{S3}]$$

$$\begin{aligned} y &= n \left(c(\beta_1\delta_2 - \beta_2\delta_1) + \beta_1^2 \frac{\partial^2 \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{1,p}^2} \right. \\ &\quad \left. + 2\beta_1\beta_2 \frac{\partial^2 \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{1,p} \partial F_{2,p}} + \beta_2^2 \frac{\partial^2 \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{2,p}^2} \right), \quad [\text{S4}] \end{aligned}$$

where c is a positive constant. To obtain Eqs. S3 and S4 we use that $\partial \rho / \partial F_1 = -c\beta_2$ and $\partial \rho / \partial F_2 = c\beta_1$. These equalities can be derived in the following way. Eq. 3 in the main text, specifying the value of the critical point θ^* in the constrained trait space, can be rewritten as

$$0 = \frac{\partial p}{\partial F_1} \beta_1 + \frac{\partial p}{\partial F_2} \beta_2.$$

This equality is equivalent to $(\partial \rho / \partial F_1) / (\partial \rho / \partial F_2) = -\beta_2 / \beta_1$, which implies $\partial \rho / \partial F_1 = -c\beta_2$ and $\partial \rho / \partial F_2 = c\beta_1$ for some positive constant c .

In the following we are interested in the dominant eigenvalue and the corresponding eigenvector of the Hessian matrix. The first one allows us to determine whether the point (θ^*, θ^*) is a saddle point whereas the latter corresponds to the orientation of a potential saddle point in the (θ_1, θ_2) plane. The eigenvalues of the Hessian matrix equal $\lambda_{1,2} = n/2(a \pm \sqrt{b})$ with

$$a = x + (p^2 + (1-p)^2)y \quad [\text{S5}]$$

$$b = ((1-2p)(x+y))^2 + 4p^2(1-p)^2y^2. \quad [\text{S6}]$$

Four cases can be distinguished:

- i) $a > 0$ and $b > a^2 \Leftrightarrow \lambda_1 > 0$ and $\lambda_2 < 0$ (saddle point)
- ii) $a > 0$ and $b < a^2 \Leftrightarrow \lambda_1 > 0$ and $\lambda_2 > 0$ (maximum)
- iii) $a < 0$ and $b > a^2 \Leftrightarrow \lambda_1 > 0$ and $\lambda_2 < 0$ (saddle point)
- iv) $a < 0$ and $b > a^2 \Leftrightarrow \lambda_1 < 0$ and $\lambda_2 < 0$ (minimum).

We are interested in the situation where (θ^*, θ^*) is a saddle point of the fitness landscape; i.e., $\lambda_1 > 0$ and $\lambda_2 > 0$. This is the case if and only if $b > a^2$. It is easy to show that

$$b > a^2 \Leftrightarrow 0 > 4(1-p)px(x+y). \quad [\text{S7}]$$

We are interested in the situation where the point (θ^*, θ^*) is not only a saddle point but simultaneously a maximum in the constrained trait space. The curvature at (θ^*, θ^*) in the direction of the constrained trait space is given by $(1/\sqrt{2}, 1/\sqrt{2})H(1/\sqrt{2}, 1/\sqrt{2})^T$.

Evaluating this expression shows that it is negative if and only if $x + y < 0$. Then, provided that $x + y < 0$, inequality Eq. S7 is fulfilled if and only if $x > 0$. To summarize, $-y > x > 0$ is necessary and sufficient for (θ^*, θ^*) to be a saddle point of the fitness landscape while simultaneously being a maximum in the constrained trait space. Condition Eq. 6 in the main text is recovered by undoing the substitutions $\partial\rho/\partial F_1 = -c\beta_2$ and $\partial\rho/\partial F_2 = c\beta_1$ in x . Then $x > 0$ equals condition Eq. 6. This condition is therefore not restricted to the case of two modules but applies to any number of modules and any allocation pattern of modules over the two tasks.

We continue by determining the dominant eigenvector of the Hessian matrix. This vector calculates to $u_d = (1, u_{d2})$ with slope

$$u_{d2} = \frac{2p(1-p)y}{(1-2p)(x+y) - \sqrt{b}}, \quad [\text{S8}]$$

where b is given by Eq. S6. Above we showed that (θ^*, θ^*) is a saddle point if $0 < x < -y$. For $x = 0$ the dominant eigenvalue of the Hessian matrix equals zero and the slope simplifies to $u_{d2} = -p/(1-p)$. Thus, for $x = 0$ phenotypes $(\theta^* + \Delta_1, \theta^* - \Delta_2)$ (where Δ_1 and Δ_2 are small and positive) with

$$\frac{\Delta_2}{\Delta_1} = \frac{p}{1-p} \quad [\text{S9}]$$

have the same fitness as the phenotype (θ^*, θ^*) whereas all other phenotypes have lower fitness. Solving Eq. S9 for p gives $p = \Delta_2/(\Delta_1 + \Delta_2)$, which can be rewritten as Eq. 11 in the main text. In conclusion, if x is just marginally larger than zero, i.e., if condition Eq. 6 in the main text is just barely fulfilled, then functional specialization can evolve if genetic variation exists in the direction of the vector $(1, -p/(1-p))$.

In the following we investigate several important features of the saddle point as x increases from zero to $-y$. First, the dominant eigenvalue changes according to

$$\frac{d\lambda_1}{dx} = \frac{n}{2} \left(1 + \frac{(x+y)(1-2p)^2}{\sqrt{b}} \right).$$

It can be shown that the equation $d\lambda_1/dx = 0$ has no solution in the real numbers. Thus, $d\lambda_1/dx$ never changes sign and λ_1 is either monotonically increasing or decreasing as a function of x . Because for $x = -y$ we have $d\lambda_1/dx = n/2 > 0$, it follows that λ_1 is monotonically increasing in x .

Second, the slope of the dominant eigenvector changes according to

$$\frac{du_{d2}}{dx} = \frac{2p(1-p)(1-2p)y}{-(x+y)(1-2p)\sqrt{b} + b}.$$

Using that (θ^*, θ^*) is a maximum in the constrained trait space, i.e., $x + y < 0$, we see that the denominator is always positive. The sign of the numerator is determined by p and y . For $y < 0$ we find

$$\begin{aligned} \frac{du_{d2}}{dx} &< 0 \text{ for } p < \frac{1}{2} \\ \frac{du_{d2}}{dx} &> 0 \text{ for } p > \frac{1}{2}. \end{aligned}$$

In both cases, with increasing x the dominant eigenvector is tilted in the direction of the vector $(1, -1)$. Once $x = -y$ we have $(1, u_{d2}) = (1, -1)$. As noted above, for $x > -y$ the point (θ^*, θ^*) ceases to be a maximum in the constrained trait space and turns into a fitness minimum.

Third, the directions of the zero-contour lines of a saddle point can be found by solving $(1, u)H(1, u)^T = 0$ for u . This equation has two solutions:

$$u_{1,2} = \frac{py(1-p) \pm \sqrt{-(1-p)px(x+y)}}{-(1-p)(x+(1-p)y)}.$$

Differentiating u_1 and u_2 with respect to x gives

$$\frac{du_{1,2}}{dx} = \frac{py(\mp((x+y)(1-p)-px) + 2\sqrt{-(1-p)px(x+y)})}{2(x+y(1-p))^2\sqrt{-(1-p)px(x+y)}}.$$

For $x = 0$ we have $u_1 = -p/(1-p) = u_2$ whereas for $x = -y$ we have $u_1 = 1 = u_2$. Furthermore, it is easy to show that u_1 is monotonically increasing in x . For u_2 the situation is slightly more complicated. It is monotonically decreasing in x until $x = -(1-p)y$, where u_2 as a function of x has a pole. Here u_2 changes sign but continues to be monotonically decreasing in x . Thus, as x increases from zero to $-y$, the width of area where phenotypes have fitness higher than $\rho_{n,p}(\theta^*, \theta^*)$ is monotonically increasing until the saddle point turns into a fitness minimum.

These results can be summarized as follows. For $x = 0$, variation in the direction of the vector $(1, -p/(1-p))$ is neutral and all other variation is selected against. As x increases, the fitness advantage of variants that occur in the direction of the dominant eigenvector increases continuously and the direction of the dominant eigenvalue approaches $(1, -1)$. Simultaneously, the range of possible directions in the (θ_1, θ_2) plane where functional differentiation is favored becomes wider. In conclusion, as x increases from zero to $-y$, condition Eq. 11 in the main text becomes less stringent. Fig. S1 shows fitness landscapes for an organism consisting of 10 modules. Fitness landscapes are shown for three different values of p and parameters are such that x is just marginally larger than zero.

We conclude our analysis of the case of n equivalent modules by investigating how the optimal allocation of modules to the different tasks depends on x . First, we note that the curvature of the fitness landscape in the direction of a vector $(1, u)$ is proportional to

$$(1, u)H(1, u)^T = n \left(x(p + u^2(1-p)) + y(p + u(1-p))^2 \right). \quad [\text{S10}]$$

Differentiating the right-hand side of Eq. S10 with respect to p and solving the resulting expression after setting it equal to zero for p gives

$$p^* = -\frac{x + ux + 2uy}{2(1-u)y}.$$

The value p^* gives the allocation pattern that maximizes fitness for a given vector $(1, u)$. Differentiating p^* with respect to x gives $-(1+u)/2y(1-u)$. Thus, p^* increases with x for $0 > u > -1$ and decreases with x for $u < -1$. In both cases, for $x = -y$ we find $p^* = 1/2$. We can conclude that with increasing x the optimal allocation pattern of modules to the different tasks approaches $p = 1/2$ regardless of the direction of functional specialization as given by $u = -\Delta_2/\Delta_1$.

SI Text B

Extension to n Modules: Nonequivalent Modules. In the case of nonequivalent modules we allow for module-specific effects on performance. This assumption is implemented into Eq. S2 by replacing $\beta_i(\theta^* - \theta_1)$ and $\beta_i(\theta^* - \theta_2)$ with $\beta_{i1}(\theta^* - \theta_1)$ and $\beta_{i2}(\theta^* - \theta_2)$, respectively, and by replacing $\gamma_i(\theta^* - \theta_1)^2$ and $\gamma_i(\theta^* - \theta_2)^2$ with $\gamma_{i1}(\theta^* - \theta_1)^2$ and $\gamma_{i2}(\theta^* - \theta_2)^2$, respectively. With some abuse of notation we use both $\rho_{n,p}(\theta_1, \theta_2)$ and

$\rho_{n,p}(F_{1,p}, F_{2,p})$. The gradient $D\rho_{n,p}(\theta_1, \theta_2)$ of the fitness function evaluated at the point (θ^*, θ^*) has the entries

$$\frac{\partial \rho_{n,p}(\theta_1, \theta_2)}{\partial \theta_1} = -np \left(\beta_{21} \frac{\partial \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{2,p}} + \beta_{11} \frac{\partial \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{1,p}} \right) \quad [\text{S11a}]$$

$$\frac{\partial \rho_{n,p}(\theta_1, \theta_2)}{\partial \theta_2} = -n(1-p) \left(\beta_{22} \frac{\partial \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{2,p}} + \beta_{12} \frac{\partial \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{1,p}} \right). \quad [\text{S11b}]$$

The derivative of the fitness function in the direction of the constrained trait space, $d\rho_{n,p}(\theta, \theta)/d\theta$, is equal to the sum of the expressions on the right-hand side of Eqs. **S11a** and **S11b**. At θ^* we have $d\rho_{n,p}(\theta, \theta)/d\theta = 0$, which implies

$$\frac{\partial \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{1,p}} = c(p(\beta_{22} - \beta_{21}) - \beta_{22}) \quad [\text{S12a}]$$

$$\frac{\partial \rho_{n,p}(F_{1,p}, F_{2,p})}{\partial F_{2,p}} = c(p(\beta_{11} - \beta_{12}) + \beta_{12}) \quad [\text{S12b}]$$

for some positive constant c . Inserting Eqs. **S12a** and **S12b** into $D\rho$ gives $(1, -1)$ as direction of steepest fitness increase at the point (θ^*, θ^*) . The derivative of the fitness function in the direction of the vector $(1, -1)$ equals

$$\frac{\partial \rho_{n,p}(\theta_1, \theta_2)}{\partial \theta_1} - \frac{\partial \rho_{n,p}(\theta_1, \theta_2)}{\partial \theta_2} = -2cnp(1-p)(\beta_{12}\beta_{21} - \beta_{11}\beta_{22}). \quad [\text{S13}]$$

Thus, the derivative in the direction of the vector $(1, -1)$ is positive if $\beta_{11}\beta_{22} > \beta_{12}\beta_{21}$ and negative otherwise. This finding generalizes condition Eq. 7 from the main text to the general case of arbitrarily many modules. In conclusion, with non-equivalent modules generically the point (θ^*, θ^*) is not an extremum in the extended trait space but directional selection favors differentiation such that modules predisposed for one task become even more specialized.

SI Text C

Specialization of Duplicated Genes. Performance is defined as the total amount of substrate converted and denoted $F_1(a_{1,1}, a_{2,1})$ and $F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))$. The curvature of the performance landscape for the first substrate in the direction orthogonal to the constrained trait space at a point (a^*, a^*) is given by Eq. 5 in the main text where θ_1 and θ_2 have to be replaced by $a_{1,1}$ and $a_{2,1}$, respectively,

$$C_1 = \frac{1}{2} \left(\frac{\partial^2 F_1(a_{1,1}, a_{2,1})}{\partial a_{1,1}^2} + \frac{\partial^2 F_1(a_{1,1}, a_{2,1})}{\partial a_{2,1}^2} - 2 \frac{\partial^2 F_1(a_{1,1}, a_{2,1})}{\partial a_{1,1} \partial a_{2,1}} \right), \quad [\text{S14}]$$

with all derivatives on the right-hand side evaluated at a point (a^*, a^*) . The first two terms describe whether the amount of converted substrate 1 is an accelerating or a saturating function of substrate affinity. The third term describes the effect of a possible interaction between enzymes coded by different loci. The curvature of the performance landscape for the second substrate at a point (a^*, a^*) is more complicated because, due to the manner in which we introduced the trade-off, $F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))$ is not directly a function of $a_{1,1}$ and $a_{2,1}$ but via the functions $a_{1,2}(a_{1,1})$ and $a_{2,2}(a_{2,1})$,

$$C_2 = \frac{1}{2} \left(\frac{\partial^2 F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))}{\partial a_{1,2}^2} \left(\frac{da_{1,2}}{da_{1,1}} \right)^2 + \frac{\partial^2 F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))}{\partial a_{2,2}^2} \left(\frac{da_{2,2}}{da_{2,1}} \right)^2, \right. \\ \left. - 2 \frac{\partial^2 F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))}{\partial a_{1,2} \partial a_{2,2}} \frac{da_{1,2}}{da_{1,1}} \frac{da_{2,2}}{da_{2,1}} \right. \\ \left. + \frac{\partial F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))}{\partial a_{1,2}} \frac{d^2 a_{1,2}}{da_{1,1}^2} + \frac{\partial F_2(a_{1,2}(a_{1,1}), a_{2,2}(a_{2,1}))}{\partial a_{2,2}} \frac{d^2 a_{2,2}}{da_{2,1}^2} \right), \quad [\text{S15}]$$

with all derivatives on the right-hand side evaluated at a point (a^*, a^*) . Using that at (a^*, a^*) for equivalent modules $\partial F_1/\partial a_{1,1} = \partial F_1/\partial a_{2,1}$, $\partial F_2/\partial a_{1,2} = \partial F_2/\partial a_{2,2}$, $\partial^2 F_2/\partial a_{1,2}^2 = \partial^2 F_2/\partial a_{2,2}^2$, $da_{1,2}/da_{1,1} = da_{2,2}/da_{2,1}$, and $d^2 a_{1,2}/da_{1,1}^2 = d^2 a_{2,2}/da_{2,1}^2$ we find condition Eq. 9 in the main text.

Next we consider the case that enzymes are dimers. By $a_{i,kl}$ we denote the affinity of an enzyme for the i th substrate where the dimer consists of one gene product coded by the k th locus and one gene product coded by the l th locus ($k, l \in \{1, 2\}$). We investigate the effect of specialization under the assumption that both loci specialize to an equal extent but for the alternative substrates (i.e., $a_{i,11} - a^* = a^* - a_{i,22}$), thus, in the direction where specialization is most likely to be favored by selection. Our treatment is based on the assumption that the affinity of heterodimers, $a_{i,12}$, can be described as a function of the substrate affinity of the two corresponding homodimers, $a_{i,11}$ and $a_{i,22}$. More specifically, sufficiently close to the point (a^*, a^*) we approximate $a_{i,12}$ by

$$a_{i,12} = a^* + \beta_{i,12} (|a_{i,11} - a_{i,22}|) + \frac{1}{2} \gamma_{i,12} (a_{i,11} - a_{i,22})^2. \quad [\text{S16}]$$

Performance functions are approximated by

$$F_i(a_{i,kl}) = F_i(a^*) + \beta_i (a_{i,kl} - a^*) + \frac{1}{2} \gamma_i (a_{i,kl} - a^*)^2. \quad [\text{S17}]$$

After inserting Eq. **S16** into Eq. **S17** and neglecting terms of order higher than two we can, with some abuse of notation, write performance of heterodimers as

$$F_i(a_{i,11}, a_{i,22}) = F_i(a^*) + \beta_i \beta_{i,12} (|a_{i,11} - a_{i,22}|) + \frac{1}{2} \beta_i \gamma_{i,12} (a_{i,11} - a_{i,22})^2 + \frac{1}{2} \gamma_i \beta_{i,12} (a_{i,11} - a_{i,22})^2.$$

If homodimers are formed with probability p , heterodimers with probability $1-p$, and the two different homodimers are equally likely, then the average affinity equals

$$\bar{F}_i(a_{i,11}, a_{i,22}) = \frac{p}{2} F_i(a_{i,11}) + \frac{p}{2} F_i(a_{i,22}) + (1-p) F_i(a_{i,11}, a_{i,22}).$$

Performance of two differentiated loci exceeds performance of two undifferentiated loci, if

$$F_i(a^*) < \bar{F}_i(a_{i,11}, a_{i,22}), \quad [\text{S18}]$$

or, alternatively, using $(a_{i,11} - a^*) = -(a_{i,22} - a^*)$ if

$$0 < p\gamma_i(a_{i,kk} - a^*)^2 + (1-p)(2\beta_i\beta_{i,12}(|a_{i,11} - a_{i,22}| + (a_{i,11} - a_{i,22})^2(\beta_i\gamma_{i,12} + \gamma_i\beta_{i,12}))).$$

Using that $|a_{i,11} - a_{i,22}| = 2|a_{i,kk} - a^*|$, we can rewrite condition Eq. S18 as

$$0 < p\gamma_i + 4(1-p)\left(\frac{\beta_i\beta_{i,12}}{|a_{i,kk} - a^*|} + \beta_i\gamma_{i,12} + \gamma_i\beta_{i,12}\right). \quad [\text{S19}]$$

This inequality allows for the following interpretations. First, as $|a_{i,kk} - a^*|$ approaches zero, only the sign of $\beta_i\beta_{i,12}$ decides whether condition Eq. 6 is fulfilled. Here we are interested in the

case that performance increases with increasing affinity; i.e., $\beta_i > 0$. Thus, if enzymes consisting of two different gene products are superior, i.e., $\beta_{i,12} > 0$, then specialization results in increased performance. Second, if $|a_{i,kk} - a^*|$ is small but finite, $\beta_{i,12} > 0$, and $\gamma_i < 0$ (i.e., performance is, for example, due to Michaelis–Menten kinetics, a saturating function of substrate affinity), then specialization is favored if heterodimers are formed sufficiently frequently. More specifically, condition Eq. S19 can be rewritten as

$$p < \frac{1}{1 - \gamma_i/4\left(\frac{\beta_i\beta_{i,12}}{|a_{i,kk} - a^*|} + \beta_i\gamma_{i,12} + \gamma_i\beta_{i,12}\right)}.$$

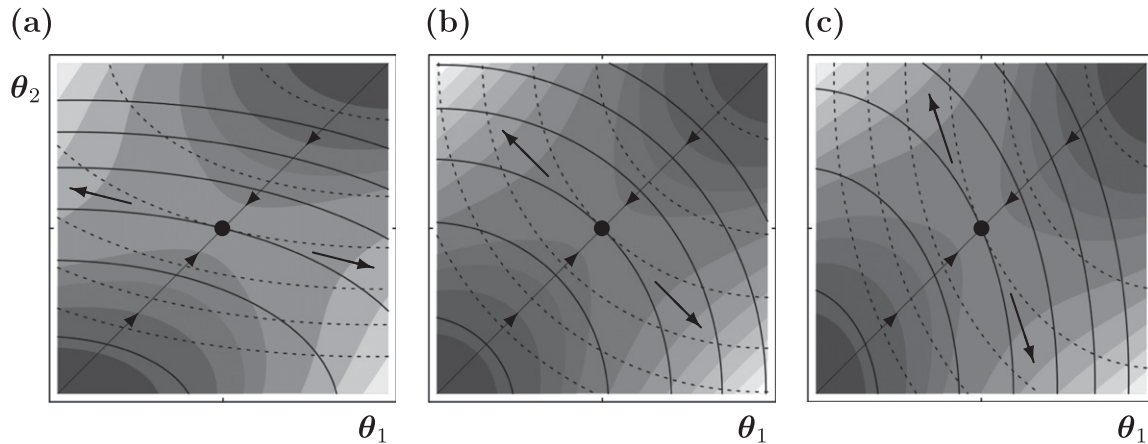


Fig. S1. Fitness landscape for the case of $n = 10$ equivalent modules, with pn modules characterized by θ_1 and $(1 - p)n$ modules characterized by θ_2 , locally around a point (θ^*, θ^*) (solid circles). Values of p equal (A) $p = 0.2$, (B) $p = 0.5$, and (C) $p = 0.7$. Shading corresponds to contours of the fitness function with lighter shades indicating higher values. The expected direction of the evolutionary dynamics is indicated by arrows. Iso-performance curves for F_1 and F_2 , introduced in the Fig. 1 legend in the main text, are shown as solid and dashed curves, respectively. Functions $F_i(\theta_1, \theta_2)$ are given by Eq. S2 and $\rho(F_1, F_2) = F_1^*F_2$. Other parameter values: $\theta_3 = 0.5$, $\alpha^1 = 1 = \alpha^2$, $\beta^1 = -10$, $\beta^2 = 10$, $\gamma^1 = 500 = \gamma^2$, and $\delta^1 = -10 = \delta^2$.