## Vertical, horizontal or environmental? Research on symbiont acquisition calls for proper controls

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Symbiotic associations are ubiquitous in nature and can have profound effect on animal diversity and evolution. Symbionts can be transmitted i) vertically, when symbionts are passed directly from mother to offspring during early life; ii) horizontally transmitted symbionts are acquired in later life-stages through feeding on infected corpses, faeces, or through physical contact between conspecifics; and iii) environmentally transmitted symbionts, also acquired later in life, can be obtained through contact with or uptake of surrounding matter such as water or soil. Many animal hosts are also associated with more than one key symbiont, and these communities and their genes that they contain are referred to as microbiomes. Majority of studies describe the microbiome in terms of bacterial diversity without knowing how microbes are acquired and what is the proportion of microbes derived from early-life colonization. We propose a method that allows to control the contribution of early-life microbial colonization in a model organism group, terrestrial isopods (Crustacea: Isopoda) that is characterized by brooding developing embryos inside the female brood pouch i.e., higher chances for mother-offspring symbiont transmission. Our experimental approach involves two steps: obtaining symbiont-naïve offspring from the brooding mother while preventing any source of horizontal and environmental transmission; and subsequently simulating alternative transmission modes through feeding manipulation and quantifying the host fitness. Our method exemplifies what is required to disrupt a vertical transmission in animal model systems with a parental brooding, and in the host organism that entail significant benefits from the acquired microbes.