as killer whales that have depredated toothfish around the Crozet Islands. As with other marine predators, killer whales around the world are likely to be impacted by ecosystem collapses caused by anthropogenic climate change.

Where can I find out more?

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DECLARATION OF INTERESTS

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Primer

Vaginas

Rachel M. Keeffe and Patricia L.R. Brennan*

What is the vagina? This seemingly simple question has a rather complex answer, depending on whether we use a functional or a developmental definition. The terminal portion of the female reproductive tract that opens to the environment initially served as a conduit for eggs to be laid, and in species with external fertilization the distal oviduct may be specialized for oviposition but there is no vagina. In animals with internal fertilization, this terminal section of the oviduct interacts with the sperm and the intromittent organ leading to functional specialization of this region that we often call a vagina in insects and some vertebrates. Here we address the evolution, morphology and diverse functions of the vagina and some of the unknown questions that remain to be addressed in the study of this remarkable structure.

In this primer, we use a functional definition of the vagina as the region of the reproductive tract that connects the genital or cloacal opening to the oviducts, and that interacts with the intromittent organ and/or sperm during copulation. The use of this functional definition of vagina avoids the confusion brought about by lack of agreement on the use of the term (Table 1). In non-vertebrate multicellular animals, the entrance to the female reproductive tract can be complex, and have more than one opening, followed by a vagina that can have either a soft or a chitinized/cuticular inner surface, that often leads directly to a single or multiple *bursa copulatrix*, and/or spermathecal ducts. Sometimes a separate vagina is absent, and the intromittent organ enters the *bursa copulatrix* or the spermathecal ducts directly.

Most vertebrates have a single cloaca, a chamber where the terminal portions of the intestine and urogenital tracts come together and exit the body through a single opening, which often becomes specialized for a copulatory function. Yet many anatomists do not use the word ‘vagina’ to describe this region despite its functional specialization that distinguishes it from the male cloaca, and many other synonyms can be found in the literature. In marsupial and placental mammals, which have distinct urogenital and anal openings as opposed to a single cloaca, there is anatomical agreement as to the use of the word vagina to describe the region of the urogenital canal that connects the opening to the cervix. Male cave insects and seahorses are often described as having a vagina because they receive female eggs into their reproductive tracts, and the most distal region of the male reproductive tract interacts with the female intromittent organ.

Evolution and function

The vagina has multiple important functions that include protecting the upper female reproductive tract from infection, interacting mechanically with the intromittent organ during copulation either to facilitate or limit successful insemination, expanding and contracting to allow for oviposition or parturition, and healing/recovering if tissue tears have occurred while accomplishing any of these functions. The vagina also plays a role in interacting with sperm, often functioning in its transport, storage, or rejection.

The vagina evolved from oviduct tissues that directed eggs to the outside of the body to be fertilized (Figure 1). A distinct protective function against potential pathogens probably evolved early on, via mechanical closure of the female tract with sphincters, and with the evolution of immune defenses against infection that could be acquired from the external environment or from waste excretion. The urogenital opening in external fertilizers is sometimes, but not always, separate from the anal sphincter. With internal fertilization, the risk of pathogens brought in from the intromittent organ and sperm increases. This leads to further specialization of the female tract, particularly in the vagina, as the first line of immune defense. In some animals, including some arachnids, insects and mammals, the vaginal opening only appears when the female is ready to begin reproducing. This vaginal closure may further protect females from infection and from unwanted copulations that may not lead to fertilization. The separation of the cloaca into distinct urogenital and anal openings results in the novel development of a separate vagina in most mammals. In this case, the vagina was believed to develop cranially from epithelium derived from the Mullerian ducts and caudally from epithelium derived from the urogenital sinus, although recent evidence suggests only the latter is the primary tissue type involved in the development of the mammalian vaginal canal.

Other vaginal specializations include modifications for sperm storage that can be folds, crypts or just areas where sperm seem to be able to stay alive for some period of time (Figure 1). Copulation itself introduces novel mechanical disruptions that impose further selection on those tissues interacting directly with the intromittent organ. This selection can lead to mechanical specializations including increased vaginal wall thickness, higher density of connective tissue, and sclerotization of the vaginal lining. In addition, the transition from egg laying to parturition demands additional adaptations within the vagina, considering its expansion during birth and recovery after stretching.

Table 1. Synonyms for the word vagina in different animal taxa.

Group	Synonym
Insects	Vestibule, Uterus, Bursa Copulatrix (when a separate vagina is absent), Atrium Copulatorium, Vaginal Appendix, Spermatheca (when a separate vagina is absent), Spermathecal Ducts.
Vertebrates	Pouch / Vaginal Pouch, Female Genital Channel, Urogenital Chamber, Sinus Urogenitalis, Urodeum of the Cloaca, Sexual Ducts, Introitus.
Other metazoans	Genital Chamber, Oviducal Gland, Copulatory Duct, Genital Atrium.

Many different names have been applied to vaginas, given a functional definition of the region of the reproductive tract that connects the genital or cloacal opening to the oviducts, and that interacts with the intromittent organ and/or sperm during copulation.

Tissue architecture

The disparate functions of the vagina are performed by its integrated tissue architecture, and this architecture is similar in animals with internal fertilization, comprising a luminal or most internal layer, a layer of connective tissue that stretches, and may or may not be distinct, and a muscular layer that contracts (Figure 2). In some insects, the distal, external parts of the segments that form the dorsal and ventral lips of the gonopore, or genital opening, are sclerotized or hardened. Proximal to the entrance, where those segments form the dorsal and ventral walls of the vagina, they sometimes become membranous. The tunica intima of the reproductive tract forms the innermost layer of the vagina. A thick, muscular layer surrounds the vaginal walls. Anteriorly, the oviduct and spermathecal duct (when present) open into the vagina and in some species the spermatheca is also sclerotized or covered with a cuticle.

In placental mammals (Figure 2) the most internal mucosal layer has an important immune function. The lumen is typically full of specialized secretory white blood cells, or eosinophils, that likely aid in the maintenance of the epithelium itself. This epithelium layer consists of stratified, non-keratinized squamous cells that are constantly shedding, a process that helps eliminate potential pathogens, but that also results in the formation of new tissue if the mucosa is mechanically abraded. The *lamina propria* below the epithelial tissue contains multiple elastin fibers that help to convey elasticity to the vaginal walls, allowing for extensive deformation. The *muscularis* layer below the *lamina propria* comprises smooth muscle that runs circumferentially in the most internal layer, and longitudinally in the most external layer. This muscular layer is responsible for the contractile properties of the vagina that are needed during egg laying or birthing, and sometimes during copulation. The outermost layer, the *adventitia* layer, connects the vaginal canal to the surrounding structures, including the ureters, blood vessels, bladder, and rectum, through a thick layer of connective tissue. This layer is typically arranged in sheets that keep the vagina in place, and can help prevent twisting in species where the vagina has a complex shape.

Mechanical functions of the vagina

Vaginal tissues are often under mechanical stress in many clades, but work on the mechanical tissue properties of the vagina has been mostly limited to mammals and a few insects. Mechanical stress on vaginas during copulation is likely to differ greatly across species because of the different intromittent organ morphologies and copulatory behaviors observed in nature; from the single brief intromission in ducks (less than one-third of a second), to the prolonged, hours-long affair often seen in snakes, multi-week mating in stick insects, to the many short bouts of thrusting seen in rodent copulation, to the genital lock with no thrusting observed in turtles and many beetles. Further, the presence of spines in many intromittent organs can impose extreme mechanical injury to the vaginal wall. In bats and *Callosobruchus* beetles, the thickness of the vaginal wall is increased in species where the males have spiny intromittent organs (Figure 2). Presumably, this increased vaginal thickness reduces copulatory harm to the vaginal tissue. Sclerotization of the vaginal wall in insects may also protect the vaginal wall from abrasion during copulation. However, in fruit flies, there is evidence that some seminal fluid proteins enter the female circulatory system through the ventral posterior vagina, which can be punctured by the male aedagus or intromittent organ. In this region, musculature that surrounds the rest of the vagina is absent, as well as the superficial epithelial cell layer present in other regions of the vagina, so females can sometimes adapt to potential harm by either making their vaginal tissues tougher, or by making damage fairly easy in a specific region of her tract, which likely can be healed relatively easily. There are not enough data at present to predict when females may evolve each type of adaptation.

Few studies have characterized the effects of diverse copulatory behaviors on mechanical properties of the vagina in non-laboratory models. Some beetles have an extremely long flagellum at the tip of the intromittent organ, and the females have a highly coiled spermathecal duct where the flagellum enters during copulation. Multiple

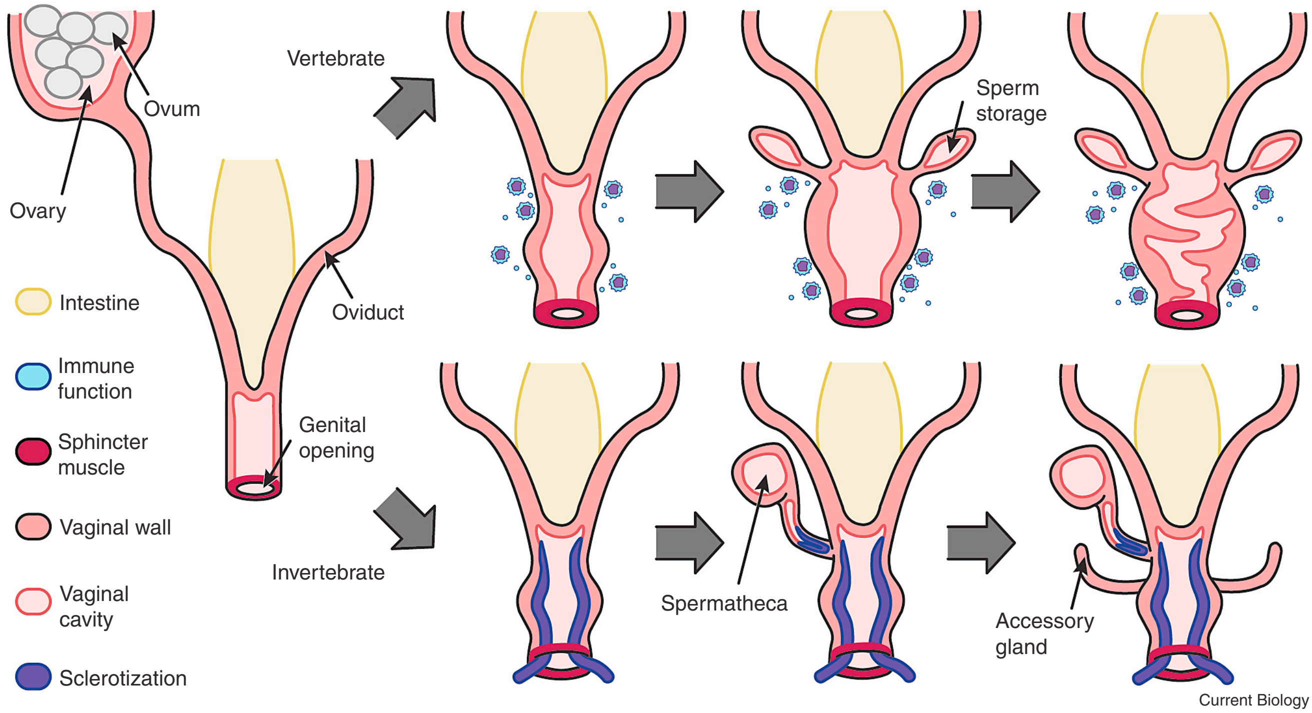

Figure 1. Evolution of the vagina.
From left to right, an illustration of the development of complex morphologies in the vagina over evolutionary time for both vertebrates (upper path) and invertebrates (lower path). Dark red indicates the cloacal sphincter, pink indicates the vaginal wall, light pink indicates the vaginal cavity, yellow indicates the intestine, dark purple indicates sclerotization, and blue and purple symbols represent immune function.

mechanical adaptations in the male and the female allow for copulatory success but the high stiffness of the female duct is critical to copulation in this system. In bottlenose dolphins, the female vaginal fold present inside the vaginal lumen is tougher and more elastic than other parts of the female reproductive tract because the penis shaft is stopped at this fold during copulation, and the fold must therefore absorb high intromittent forces.

In mammals, the vagina consists of the same three layers along its length but its mechanical properties and behavior under load vary significantly and are different in the longitudinal and circumferential directions. Complexity in mechanical behavior may be expected in other systems as the result of other functions of the vagina, not just copulatory interactions, that may require different mechanical performance. For example, oviposition varies across animals, especially regarding egg number, egg size, and oviposition frequency. Birds typically lay a single large egg every day for a few days, while fishes and many insects may lay hundreds of eggs almost simultaneously just once in their lifetime. Parturition can involve the expansion of the vagina to 225% of its original size to give birth to extremely large offspring/eggs as is the case in humans, some skates, and some birds, or it may involve almost no expansion at all to give birth to tiny precocial babies such as in marsupials. The role of these different modes of reproduction on the mechanical properties of vaginal tissues has yet to be investigated.

Injury to the vaginal wall that can occur during copulation and/or parturition may result in scarring that would cause local stiffness and result in asymmetric and/or limited tissue expansion. For example, people who experience pelvic prolapse and partial recovery have a higher incidence of recurrence due to stiffened vaginal wall tissue. Perhaps pre-adaptations to parturition or egg laying minimize the likelihood of tearing or harm to the vaginal tissues, but we know very little about how vaginal tissues behave mechanically during distinct episodes of copulation versus egg laying/parturition, and how their interaction affects vaginal function.

Vaginal shape and interactions with the intromittent organ

Vaginas are internal and often made of soft, easily deformable tissue, making the study of their functional shape difficult. The use of 2D geometric morphometric techniques that rely on repeatable points or landmarks to compare shape within and among species have revealed that snake vaginas can be significantly different among species even when they look similar to the human observer, that the rate of shape divergence in female genitalia can outpace shape changes in intromittent organs in beetles, that genital shape can constrain evolution of intromittent organs in *Drosophila*, and that phylogenetic signatures are sometimes not associated with the evolution of diverse vaginal shapes in dolphins.

Recent studies in many vertebrates have used silicone to create molds of the lumen of the vaginal canal that preserve the inside shape of the structure and are rigid (Figure 3). These molds can be made into 3D models for shape quantification, and such studies have revealed that vaginas in pregnant sharks are more asymmetrical than vaginas in non-pregnant reproductive females, that vaginas change shape as female dolphins, sharks, and snakes either become larger or enter reproductive condition, and that their shape seems to be closely coevolving with the shape of the intromittent organ in snakes. Many further discoveries are likely as comparative studies using this technique become more common.

The complex shape of the vagina has evolved either to prevent the intromittent organ from penetrating deeper into the regions where sperm are stored or used for fertilization, or to accommodate the intromittent organ during copulation. Sometimes the vaginal shape can be relatively simple and cylindrical. Even in humans, vaginal shape is largely determined by the expansion of the vaginal fornix, the most cranial region of the vaginal canal. Vaginal shape may also be influenced by whether females lay eggs or give birth, and changes during pregnancy and after parturition, but again, more studies are needed to describe these changes and how vaginas expand and recover their shape afterwards.

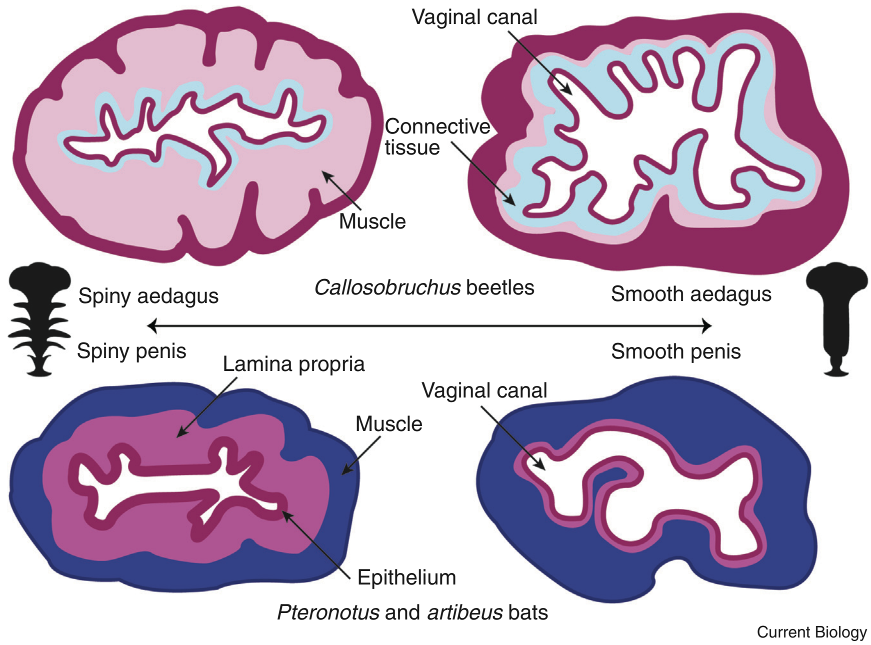

Figure 2. Vaginal tissue architecture.
Illustrated schematics representing transverse histological sections of the vaginal tissue architecture in *Callosobruchus* beetles (top row) and microchiropteran bats (bottom row). The muscular layer in *C. rhodesianus* (top left) is much thicker than in *C. phaseoli* (top right), presumably to help females avoid injury during copulation due to the presence of spines in the aedeagus. In bats, the female vaginal wall is generally thicker in species where the penis has spines (e.g. *Pteronotus parnellii*, bottom left) than in species where the penis is smooth (e.g. *Artibeus jamaicensis*, bottom right). Schematics of *Callosobruchus* beetles re-drawn from Rönn *et al*. (2007) Proc. Natl. Acad. Sci. USA *104*, 10921–10925, and those of the microchiropteran bats were referenced from Orr *et al*. (2022) Integr. Comp. Biol., icac040.

Role in immune defense

In insects, the cuticular covering of the vaginal canal is often considered the first line of immune defense via mechanical protection. In many insects, males injure the cuticular covering of the vagina during copulation, and females have evolved an immune healing response that results in melanized spots where injury took place. In *Drosophila*, antimicrobial peptides are found in the lining of the reproductive tract epithelium and act as the innate immune system.

In vertebrates, immune defense in the vagina is composed of both innate and adaptive components. In addition to mechanical closure to the external environment, antimicrobials in the thin fluid produced by epithelial and immune cells that coat the luminal surface protect against bacterial, fungal and viral pathogens by increasing the presence of specialized immune cells, known as phagocytes, that clean up organic and inorganic material, by increasing antibodies that bind bacteria, and by producing lactoferrin, a protein that lowers access to iron for bacteria. These luminal secretions also promote ciliary clearing of the vaginal lumen. Mucus production can serve as a physical barrier, and act as a trap for microbes.

The vaginal microbiome may also serve an important role in immune function. The relatively high abundance of *Lactobacillus* in the human vaginal microbiome results in an acidic pH that helps to limit the growth of pathogenic microbes, and individuals with less preponderance of *Lactobacillus* seem to be at higher risk for bacterial vaginosis, sexually transmitted infections, and spontaneous preterm birth. However, other work suggests that there is no ‘core microbiome’ of the vagina, and that vaginal microbial communities exist in a state of dynamic equilibrium that helps provide pathogen resistance.

We know little about the evolutionary changes in the vagina microbiome. The *Lactobacillus*-dominated microbiome seems to be unique to humans, as other primates do not have this microbe in abundance. The variation in microbiome composition across individuals found in humans, dogs, and primates is often related to age and estrous, and it is a barrier to characterizing a typical vaginal microbiome in most species. If the microbiome plays a role in reproductive function, then there is clearly a need for more human and animal studies that examine its composition, variation, and most importantly its function using an experimental approach. Such studies are currently rare even in laboratory models.

What studies do we need to do to better understand vaginas?

There is much that we do not know about vaginas, partly because the study of female genitalia has lagged behind the study of male genitalia for decades, even in medical settings. The perception that vaginas are somehow less variable or perhaps even less interesting than intromittent organs is demonstrably inaccurate, but this knowledge has only become available more recently with the advent of technological advances that allow us to visualize the internal, often tiny structures of the vagina.

Micro-CT scanning has allowed us to visualize the functional interaction between the vagina and the intromittent organ in preserved conjugated mating pairs in many animals. This technology has already shown that in some spiders

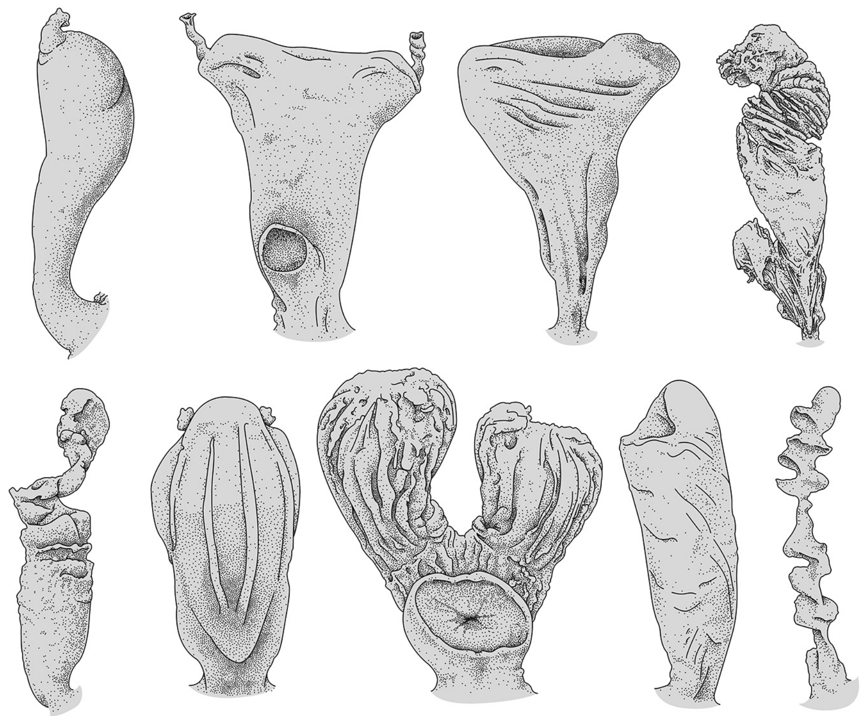

Figure 3. Morphological diversity of vertebrate vaginas.
An illustration of the morphological diversity of the vagina in vertebrates, drawn from photographs of silicone molds of the lumen of the vagina. Vaginas are drawn with the anterior end toward the top and the posterior end toward the bottom. Vaginas are not drawn to scale. Top row, left to right: alpaca (*Vicugna pacos*), winter skate (*Leucoraja ocellata*), human (*Homo sapiens*), Pekin duck (*Anas domesticus*). Bottom row, left to right: harbor porpoise (*Phocoena phocoena*), rough green snake (*Opheodrys aestivus*), spiny dogfish shark (*Squalus acanthias*), harbor seal (*Phoca vitulina*), and sperm whale (*Physeter catodon*).

inflatable vaginal structures are critical to copulatory interactions, that in dolphins vaginal folds stop penile intromission, that in *Drosophila* the intromittent organ ruptures the vaginal wall, and that in snakes the hemipenes adopt the shape of the vaginal pouch. Imaging techniques such as nano-CT scanning and X-ray tomography in insects and spiders have shown local tissue adaptation in different vaginal regions that may be punctured by spiny intromittent organs. These studies will eventually reveal important patterns of coevolution between genital structures, and provide insight as to the evolutionary mechanisms responsible for morphological diversification in vaginas and intromittent organs.

Understanding to what extent vaginal traits result from natural versus sexual selection is still a largely unexplored area of research. For example, a convoluted vagina may stop penile intromission allowing females to regain reproductive control over fertilization despite forced or unwanted copulations. However, a complex vaginal lumen could result in egg binding during egg laying, or increased newborn mortality if expanding a narrow and twisted vaginal canal is difficult. Therefore, vaginal elaboration may be limited by natural selection halting evolutionary arms races between genitalia resulting from intersexual selection processes. Comparative studies of how vaginal shape changes in species with viviparity/oviparity, and different clutch/litter size would be particularly informative to understand how these evolutionary dynamics occur.

The growing number of studies on all aspects of vaginal evolution, diversity and function are critically important to shed light on reproductive processes that have often been assumed to be under male control because of a lack of understanding of female biology. The growing body of work in this area with the advent of new technological developments is likely to result in a much richer and deeper understanding of the coevolutionary dynamics that have influenced vaginal evolution, and the role of natural and sexual selection on the underappreciated diversity of this still understudied organ.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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