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Systematic Review

Systematic Review (Up to 31 January 2025) on the Applications of Digital Organisms

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Abstract

Digital organisms (DOs) are computer-based programs designed to replicate the behaviour of biological processes, such as replication and evolution. This makes DOs a useful tool to study evolutionary processes, especially ethically challenging areas such as antibiotics resistance. Currently, there is no systematic reviews to-date examining the range of applications and studies using DOs. Here, we aim to conduct a systematic review, using studies indexed in PubMed prior to 01 January 2025, on the range of applications and studies using DOs. A total of 147 papers were identified; of which, 80 were included after screening. These 80 studies were examined by focusing on five questions; namely, (a) What was the study about? (b) What tool(s) was/were used? (c) How DOs helped in the study? (d) What was/were discovered from the studies? (e) How were findings using DOs relevant to biology? Our findings show a wide range of applications of DOs in seven main areas; namely, (i) biomechanics and movement modelling, (ii) evolutionary and adaptation, (iii) molecular and cellular evolution, (iv) ecology and community dynamics, (v) computational and synthetic systems, (vi) biomedical and environmental applications, (vii) theoretical and cross-disciplinary studies; with 31 of the 80 studies (38%) uses Avida as experimental platform.

Keywords: Digital organisms (DOs); Biology

Introduction

Digital organisms (DOs) are computer-based programs that can self-replicate, mutate, and evolve within a virtual environment [1]. They are designed to replicate how biological processes behave. This presents an opportunity to study complex behaviours in a controlled setting [2]; such as studying evolutionary biology, including Quasispecies and genetic algorithms in a controlled environment, making them valuable for evolutionary research; or simulate the population of replicating entities that evolve through mutation, allowing researchers to understand how variation within a population can affect survival and adaptation. More importantly, DOs present an opportunity to examine processes that are experimentally impossible (such as evolution from multicellularity from unicellularity) or ethically challenging areas (such as antibiotics resistance) [3].

The use of DOs has been discussed as early as the 1950s, with early computers initiating the idea of simulating life. During this decade, John von Neumann was one of the first scientists to work on self-replicating machines [4]. As technology advanced, more sophisticated DOs were developed to tackle more complex biological issues. For instance, in 1990, Tom Ray created Tierra, one of the first programs capable of simulating DOs that could evolve and rep-

licate within a virtual environment. In 1992, Avida was developed to observe evolutionary processes in real time, providing insight into how genetic variation and adaptation function [5].

Using DOs offers multiple advantages, including speed, creating precise environments, studying complex behaviours, and cost-effectiveness. DOscan evolve and adapt much faster than biological organisms, enabling researchers to observe evolutionary processes at an accelerated rate compared to conducting experiments on biological organisms [6]. Moreover, controlling the experiment's environment allows researchers to study specific variables without the complexities and unpredictability of nature. It also enables researchers to add and remove specific items from that environment, which further aids in understanding various hypotheses they might have. Additionally, DOs are significantly more cost-effective and less expensive to maintain than living organisms, as they eliminate costs associated with housing live subjects, feeding, and caring for biological specimens.

Currently there is only one systematic review on digital organisms indexed in PubMed, by Jesús Gerardo Zavala Hernández and Liliana Ibeth Barbosa-Santillán [7] examining the role in advancing Alzheimer's Disease and neurological disorders. Hence, there is no systematic reviews to-date examining the range of applications and studies using DOs. Therefore, we aim to conduct a systematic review the range of applications and studies using DOs by focusing on five questions; namely, (a) What was the study about? (b) What tool(s) was/were used? (c) How DOs helped in the study? (d) What was/were discovered from the studies? (e) How were findings using DOs relevant to biology? Our findings show a wide range of applications of DOs in seven main areas; namely, (i) biomechanics and movement modelling, (ii) evolutionary and adaptation, (iii) molecular and cellular evolution, (iv) ecology and community dynamics, (v) computational and synthetic systems, (vi) biomedical and environmental applications, (vii) theoretical and cross-disciplinary studies; with more than 38% of the studies use Avida as experimental platform.

Methods

PubMed was used to identify the studies from 01 January 1000 (default start date) to 31 January 2025 using the following search terms, (digital AND organism* AND simulation*) OR ("artificial life simulation*"). The search URL is given as (https://pubmed.ncbi.nlm.nih.gov/? term=(digital+AND+organism*+AND+simulation*)+OR+("artificial+life+simulation")&filter=dates.1000/1/1-2025/1/31). Four inclusion criteria were used; namely, (A) availability of full text, (B) articles written in English, (C) primary source, and (D) the study must be about digital organisms.

Results

A total of 147 papers were identified; of which, 80 were included after screening (Figure 1). These studies were themed into five themes (Table 1); namely, (a) What was the study about? (b) What tool(s) was/were used? (c) How DOs helped in the study? (d) What was/were discovered from the studies? (e) How were findings using DOs relevant to biology?

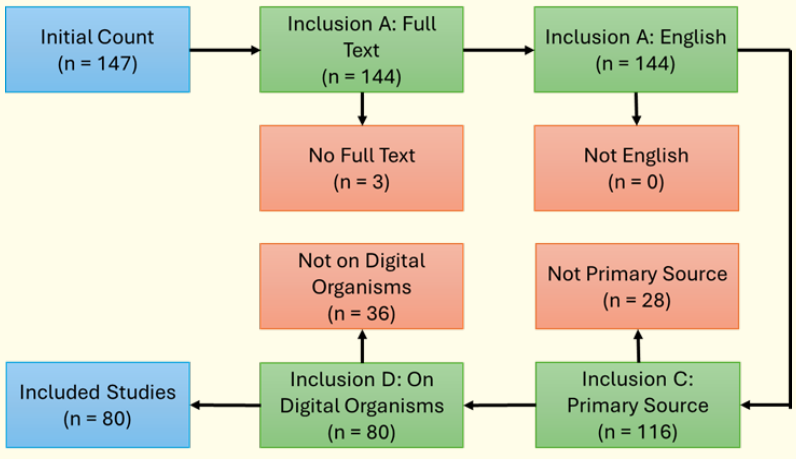

Figure 1: PRISMA Diagram Showing Screening of Articles.

Table 1: Table of Themes and Sub-Themes.

Main Theme	Sub-Theme	References
Theme 1: What is the Study about?	Biomechanics and Movement Modelling	[8-13]
	Evolutionary dynamics and genetic architecture	[1,14-49]
	Biomedical and Environmental application	[50-57]
	Microbial Ecology and Symbiosis Studies	[57-66]
	Computational and Synthetic Biology	[18,24,67-85]
Theme 2: What tools was used?	Avida	[1,15,16,21-23,26-33,33-44,55,58,59,61,62,66,67,70,71,77,79,80]
	Aevol	[46,63,75]
	GROMACS	[53,57]
	Swarm	[60,73]
	Cellular automata-based	[47,51]
	In silico models	[24,85]
	Others	[18,19,45,48-50,52,54,56,60,64,65,69,72,76,78,81-83]
	Custom	[8,11-13,20,25,68,74]
Theme 3: How digital origamis help in the study	Biomechanics and Movement Modelling	[8,10-12,60,65,73]
	Genetic and Molecular analysis	[13,18,23,24,53,54,76,82,86]
	Evolutionary dynamics and Hypothesis testing	[9,14-17,19,20,22,25-27,29-39,41-6,48,49,55,57,59,61,63,64,67,74,77,78,80,81,85,87]
	Applied Research	[15,52,56,62,69,70,72,83,84]
Theme 4: What was discovered from the studies?	Biomechanics and Movement Strategies	[8-13]
	Evolutionary dynamics and genetic architecture	[1,14-49]
	Microbial Ecology and Symbiosis	[50-57]
	Computational and synthetic biology	[57-66]
	Biomedical and Environmental applications	[18,24,67-85]
Theme 5: How are digital organisms relevant to biology?	Evolutionary Dynamics and Adaptation	[13,15-18,20-23,26,28,32-34,37,40-48,53,55,59,68,71,73,74,77,78,80,81,83,84]
	Genetic and genomic Architecture	[1,12,19,24,25,27,29,30,33,35,36,38,39,49,67,69,70,75,82,85]
	Ecological and Population Dynamics	[9,10,31,50,56,60-66,72]
	Biomedical Applications	[8,11,14,51,52,54,57,58,76,86]

Theme 1: What was the study about?

The papers explore multiple areas of interest; such as biomechanics and movement modelling, evolutionary and adaptation, molecular and cellular evolution, ecology and community dynamics, computational and synthetic systems, biomedical and environmental applications, theoretical and cross-disciplinary studies.

Five studies focus on biomechanics and movement modelling [8-13]. Frixione., *et al*. [8] investigate the quantification of *Toxoplasma gondii*'s 3d gliding, bending, and rotation, which are keys to host invasion, using kinematic simulations of flexing dynamics. Gibson., *et al*. [9] study about the feeding mechanisms of the extinct group Archaeocyatha. Hamlet., *et al*. [10] examine on how jellyfish optimise nutrient intake through bell pulsations with oral

arm contraction in stagnant water. MacIver and Nelson [11] investigates how knifefish use electro-sensors to capture prey in darkness through movement tracking. Mogg and Leguizamon [12] examines how molluscs' energy-efficient feeding locomotion aligns with Rashevsky's design principle of adequate design. Villoutreix., *et al*. [13] propose a model that integrates processes from cellular to organismal levels in sea urchin embryogenesis.

Evolutionary dynamics and genetic architecture accounts for 37 studies [1,14-49]. Two studies [14,34] track the evolution of digital populations using genotype visualisation while another [30] shows how natural selection improves the accuracy of inferred phylogenies. Researchers also investigated mutation and robustness; for example, a study examines how low-impact mutation affects long-term evolution [22] while three studies [37,38,40] examine how a high mutation rate increases robustness [38] through natural mutation or efficient selection [36,37,40] and Quasispecies theory [43]. Five studies examine fitness landscapes, such as how organisms' survival in rugged landscapes [23,39,47], genetic integration [35], and RNA antagonistic epistasis [24] impact the DOs adaptation. Studies investigated the genome's role in buffering mutations and exposing vulnerabilities [1], and how environmental pressures drive gene network evolution. Explored protein complex formation due to duplication [18] and how canalisation [19] maintains genome stability while promoting adaptability have also been explored. Several studies dive into multiciliary evolution, such as how somatic cells protect DNA from metabolic damage [21] and how larger organisms can evolve specialised cells faster [45]. Increased polyploidy enhances survival in extreme environments [25] while cellularity co-evolves with metabolic efficiency under selective resource availability pressures [48]. Studies also studied how different environmental influences, such as cyclic environments [33], population size [43], functional niches [44], and sexual reproduction [28], affect the evolvability in DOs. The researcher also investigated the different complexities, such as MADB biasing, the raptures [31], self-replicating fitness landscapes [26], optimised triplet coding, incremental EQU/odometry [1,17], and increases paradoxically where simplicity should dominate [46]. Studies also looked into how genotype-phenotype mapping [29], ontogeny recapitulation [15], historical contingency [41] and lost traits such as EQU [42] influence the evolvability and development in DOs. Lastly [27,32] it dives into the adaptive decay [27] of unused traits and how, during mating, good genes [32] are displayed.

Studies [50-57] show how DOs play a role in biomedical and environmental applications. For example, how healing relies on evolution-honed, orchestrated cell dance [51], how resistant microbes persist stubbornly post-treatment [52], how air pollution may accelerate Alzheimer's by clumping amyloid proteins [53], and *Mycobacterium tuberculosis* prioritises survival over growth under stress [54]. Canino-Koning., *et al*. [33] explain about how once-dangerous mutations may enhance a species' survival. However, ecosystems face modern stress [55] that hinders them from fully flourishing. Innovation like wastewater technology [50] and building with "Smart skins" [56] can mimic nature's efficiency to reduce energy use.

Studies [57-66] dive into microbial ecology and symbiosis studies. A study [61] dives into the cross-feeding symbiosis between DOs, showing how oscillation and equilibrium are affected by mutations. The difference between pulse vs press extinction is tested [62,66], which tests how fast communities can recover. Goldsby., *et al*. [59] explore about how the division of labour emerges when group-level selection outweighs individual fitness pressures. Fortuna., *et al*. [58] examine how host-parasite networks were able to become complex through repurposing non-adaptive traits. Ichimura., *et al*. [60] study the altruism between army ants that allows them to build bridges. Vostinar and Ofria [65] show that mutualisms flourished due to strong vertical transmission, where offspring inherit symbionts, and spatial structure [63]. Bulky populations supported cooperative patches more effectively than slender populations. Rocabert., *et al*. [64] examine th seasonality stabilised bacterial cross-feeding, unlike chemostats. Yin., *et al*. [57] demonstrate that bioluminescence imaging (KNN-BSBL method) improved tumour reconstruction accuracy using spatial data.

A group of studies had focused on the computational and synthetic biology using DOs, these studies, [18,24,67-85]. Bullinaria [71] studies imitative versus direct learning on artificial life, while research on virtual CPU architectures [70] tests how instruction

sets shape evolutionary potential. Adami., *et al*. [67] link coloured network motifs to functional complexity in digital, and LaBar and Adami [80] study population size versus complexity, and both complexity and genetic drift as a function of learning strategy (imitative vs direct) and population size. Tempesti., *et al*. [83] study biological repair mechanisms and gene arrangement through simulations. One study [75] shows how operon sharing stabilises cooperation against changes. Some studies also reveal the evolutionary trade-off by examining metabolic signalling [79], extended phenotypes [74], and parasite resistance [78]. Different studies dive into the cost of pleiotropy by using alignment tools [69] and phenotypic plasticity [81]. Researchers also simulated algae [73]; arsenic removal, and quorum sensing between bacteria [85]; and T4 phage assembly [82] to test protein self-organisation. Researchers also looked at different tools and models to bridge computational and biological systems. Bergmann., *et al*. [68] ensure reproducibility by building data.. Genoud., *et al*. [76] use Boolean logic to map Arabidopsis defence pathways in plants, while Chew., *et al*. [72] look into multi-scale simulations that helped to balance ion channels. Uriagereka., *et al*. [84] test recursion in animal communication (e.g., Fibonacci patterns) while Knoester and McKinley [77] examine self-organising behaviours (e.g., firefly-like flashing) under group selection.

Theme 2: What tool(s) was/were used?

Researchers used many types of computational platforms to simulate different biological and evolutionary processes across the 80 studies. Avida [1,15,16,21-23,26-44,55,58,59,61,62,66,67,70,71,77,79,80] is the most widely used, with 39 studies using it. Avida enables open-ended Darwinian evolution, tracking mutation rates, fitness landscapes, and novel traits like antibiotic resistance or multicellularity. Aevol [46,63,75] is used in studies on genomic evolution, focusing on cooperation and metabolic specialisation. Tierra [78] is used explore parasite resistance in self-replicating programs, while Swarm [60,73] simulated emergent behaviours like ant altruism. DOSE [52] is used to test antibiotic resistance dynamics. Other tools included Picbreeder [19], evolving images via neural networks; Evo2Sim [64], simulating bacterial interactions; MFA [82], modelling phage proteins; and FPGAS [83], enabling self-repairing circuits. Cellular automata paired with genetic algorithms [47,51] optimised evolutionary dynamics, RISER [69], Symbulation [65], Pykaryotes [18], GROMACS [53,57], TRNSYS [56] and DISCOS [45] address RNA sequencing, host-symbiont dynamics, protein evolution, and multicellularity. The floc model tool [50] simulates microbial communities in wastewater treatment systems, while genome-scale metabolic models (GMM) [54] analyses *Mycobacterium tuberculosis* metabolism under antibiotic stress using Flux Balance Analysis (FBA). Boolean network models [76] simplify plant signalling pathways into binary states to study complex regulatory behaviours. CFD [9,10] simulations analyse fluid flow in ancient organisms, while Rhinoceros 3d [11] tracks fish movement.Eight studies [8,11-13,20,25,68,74] use custom software for parasite motion analysis or modular robot evolution, including Codonevo [49], which simulates genetic coding system evolution, and artificial life (Alife) frameworks for evolving modular robots [81]. One study also uses video microscopy to record parasite movements [8]. Two studies [24,85] use an in silico model to simulate RNA secondary structure folding and to study the evolution of quorum sensing strategies. The study [84] omits computational tools and instead focuses on experimental tools such as language evolution frameworks. These platforms collectively highlight the versatility of digital systems in probing evolutionary mechanisms, from genetic drift to complex trait emergence.

Theme 3: How digital organism help in the study?

DOs have played a key role in helping researchers in their studies in biomechanics and movement modelling, genetic and molecular analysis, evolutionary dynamics and hypothesis testing, and applied research.

DOs help researchers simulate, analyse, and predict complex biological movements by tweaking different variables, such as environmental conditions and biomechanical forces [8,10-12,60,65,73]. For example, one study uses digital models to investigate how *T. gondii* moves [8]. This allows researchers to avoid the hassle of measuring live parasites, which can take up a lot of time. One paper used DOs to build a 3D fish model to track every movement without markers [11]. Researchers were able to simulate how jellyfish and molluscs move when hungry, helping them understand the biomechanics of these animals [10,12]. At the same time, researchers used digital algae to study sorption, something challenging to study in real cells due to the complexity of the cellular environment [73]. Meanwhile, another paper showed how DOs helped them understand the teamwork between ants. This was done by modelling the ants' bridge-building work [60].

DOs simplify genetic and molecular analysis by accelerating mutation studies and cutting through biological noise [13,18,23,24,53,54,76,82,86]. For example, two studies simulated evolution by adjusting mutation rates, revealing how complex protein interactions emerge, something impractical in live systems due to the complexity and difficulty of measuring and observing the changes [18,50]. Researchers were also able to blend digital embryos with biomechanics to predict how cell-level quirks (like adhesion) shape sea urchin development [13]. In another study, researchers used DOs to map antagonistic epistasis in RNA folding [24], while [54] mimicking *M. tuberculosis* metabolism in silica to test antibiotic responses without experimental laboratory. Scientists were also able to model plant signalling networks to show how they process environmental stress [76]. Researchers also simulated how air pollutants might affect Alzheimer's by promoting amyloid-beta clumping proteins [53]. Lastly, DOs are used to bridge biology and computation and decode self-assembly in artificial life [82]. These tools turn theoretical questions into testable scenarios, dodging real-world experimental headaches.

DOs let researchers tackle evolutionary questions with precision and scale, offering full control over experiments that would be messy, expensive, or downright impossible in real life [9,14-17,19,20,22,25-27,29-31,33-39,41-46,48,49,55,57,59,61,63,64,67,74,77,78,80,81,85,87]. Scientists were able to enable rapid evolution simulations without lab constraints by adjusting variables such as mutation rates. Three studies modelled feeding strategies in controlled settings while exploring how harmful mutations drive different adaptations [9,71,87]. Three studies used DOs to explore the genotype and phenotype relationships in various biological systems and map how genetic robustness influences trait evolution [29,35,75]. In four studies, DOs were used to track mutations in real time, observe complex behaviours, and document different specialisations and genetic decay [27,30,39,77]. In a study, researchers reconstructed an evolutionary family tree using DOs, allowing them to track the entire evolutionary history of different organisms, simulate real-world biology, and reveal how natural selection distorts evolutionary histories [20]. Three papers used DOs to help study evolutionary processes by allowing researchers to replicate and mutate the DOs. This allowed them to simulate biological evolution that would be similar to real life, allowing them to have a detailed overview of how mutations affect the evolutionary process [14,17,25]. Three studies used DOs to track mutation, environmental control, and genotype-phenotype analysis, enhancing scalability and reproducibility in studying evolutionary processes [21,51,81]. Other studies use DOs to test evolutionary "what-if" scenarios [25], simulate the effects of genome duplication [1,74], model trait-sharing under pressure [16,79], increased mutation rates and observing labour division akin to real-world specialisation [44,58,59,67] to track long-term adaptation dynamics, testing division of labour hypotheses across generations. In one of the papers, DOs also helped to model sexual selection through runaway evolution [32], while another paper used DOs' ability to do multiple replicates to investigate the different evolutionary dynamics across 12 populations over 500 thousand generations, which would have been virtually impossible within reasonable in a wet laboratory [64]. Researchers also used DO to test post-extinction population recovery through mutation/resource shifts [66]. Four studies employed DOs to investigate the role of genetic robustness in facilitating evolutionary innovations within existing fitness landscapes to examine adaptation [36,38,63,80]. In four other studies, DOs also played a key role in helping to track how mutations impact the cyclical environments [26,33,34,40]. In one study, DOs helped to mimic RNA/Protein interactions over thousands of generations, which allowed researchers to observe how the codon length changes over different generations [49]. DOs were also used to study the transition from non-cellularity to cellularity. This was done by measuring different things, such as coevolutionary relationships and how metabolism and cell influence each other [48]. Another paper used DOs to help simulate tumour structure [57]. Three papers used DOs to study the viral quasi-species and their role in evolution [38,43,46]. Lastly, one of the papers uses DOs to help test for parasite resistance by tracking different mutations [78].

Applied research was another area that uses DOs [15,52,56,62,69,70,72,83,84]. Tempesti., *et al*. [83] develop hardware self-repair models using simulations. Borozan., *et al*. [69] simulate human/viral RNA sequences to test pathogen-detection algorithms and analyse mutations. One study showed how DOs helped model mass extinctions to study their ecological impacts on the environment [62]. Another study used DOs to help design bio-inspired building facades that mimic animal fur and blood persuasion [56]. DOs also helped researchers to evolve communication systems to study language structure by changing different pa-

rameters [84]. Researchers also used DOs to help combine carbon dynamics and gene networks to predict the growth of Arabidopsis [72]. DOs also allowed researchers to control the evolutionary environments, removing cofounding factors and using more than 50 independent runs to provide unbiased data [15]. In one paper, DOs were experimented using different CPU designs to test the impact on evolutionary adaptability [70]. Lastly, DOs helped to track antibiotic resistance evolution under different selection pressures [52].

Theme 4: What was/were discovered from the studies?

In the 80 studies, multiple new breakthroughs were achieved in areas; such as biomechanics and movement strategies, evolutionary dynamics and genetic architecture, microbial ecology and symbiosis, computational and synthetic biology, and biomedical and environmental applications.

Studies have [8-13] uncovered how organisms tweak their posture, lean on sensory cues, and fine-tune their movements, almost like engineers, to hunt and navigate, all without a central control system (DOs). Digital simulations were able to decode different biomechanical puzzles, such as fluid dynamics in extinct species. This showed a discovery of suspension feeding [9]. Researchers were also able to discover the machine-like spins fuelled by actin-myosin teamwork and twisting microtubules [8] found in parasites. They also found out how electric fish use electro-sensory intel to stalk prey and make real-time adjustments to catch them [11]. Studies also showed how Jellyfish [10] have evolved a clever trick with fluid dynamics: their sieve-like oral arms manipulate water flow to improve how they feed. A study [12] shows the different strategies that reflect evolutionary niche specialisation, balancing energy conservation, fluid mastery, and sensory precision. A shared biological playbook, including helical microtubules and actin networks, allows parasites to store elastic energy and generate torque. Development also adheres to rules; for example, blastula formation follows cell cycle timing, not just a rigid "mitotic gradient", to guide cell division [13].

Multiple studies showed how DOs have helped to discover new things in evolutionary dynamics and genetic architecture by tackling traditional limitations like slow timescales, complex genetic interactions, and inaccessible scenarios [1,14-49]. In one study, researchers discovered how polyploidy is beneficial in harsh environments [25]. Two studies revealed the hierarchical genetic architecture that showed how altruistic behaviour emerges naturally [19,20]. Another study found that organisms with higher mutation rates can outcompete organisms with a slower replicating rate [15,23]. Researchers also validated the adaptive trade-offs of the "Dirty Work Hypothesis" and exposed extremophile/parasite evolutionary strategies [16]. Natural selection improves phylogenetic accuracy via lineage-specific rate variation (ASRV) over uniform models [30], with fluctuating environments amplifying adaptive signals [41]. Mutation bias in traits that inform astrobiology [31], while cyclic environments boost evolvability compared to static fitness optimisation [33]. Adaptation retains genetic memory shaped by mutation rates and environmental stress [34]. Studies link fluctuating environments' cost of sex to Red Queen dynamics [28], while robustness/genotype networks trade adaptability for evolvability [29,36]. Hypermutation drives neutral genome restructuring [69], exposing fitness plateaus [30]. Neutral mutations reduce harmful epistasis, though robustness challenges neutrality assumptions: fit genotypes exploit positive epistasis; "flat" ones dominate [37,43]. In one paper, DOs helped to discover how mutation rates evolve at a sub-optimal level in a selection of rugged landscapes [40]. In two studies, researchers found out that larger organisms evolve specialised cells much faster than smaller ones because the mutations in larger cells are less detrimental [44,45]. In another paper, a researcher discovered that DOs would completely irreversibly increase [46]. Two papers showed how organisms go through enhanced robustness despite the fitness cost [1,47].

A number of studies [57-66] show how DOs helped discover new things in microbial ecology and symbiosis. They compress years of observation into days, revealing hidden dynamics such as cross-feeding and biofilm formation [61, 85]. Lab experiments, however, are still essential but computational models can predict things such as extinction or metabolic collapse, which can be used to guide real-world trials. One example is the cross-feeding studies [61], which show how microbes evolved mutual dependencies through metabolic byproducts, with mutations stabilising partnerships. Sudden changes in the environment, as shown in *Escherichia coli* [62,66] experiments, upset stability. In contrast, gradual "pulse extinctions" help ecosystems become more resilient. However, a slow environmental decline can leave ecosystems in an unhealthy state. Symbiosis study [58] also reveal how host-parasite systems are able to repurpose traits for defence, while microbial clustering [65] curbs selfish "cheaters".

Ant colonies [60] can adjust their altruistic behaviours through group competition [59]. Environmental volatility complicates this: cyclical scarcity preserves diversity, but extreme stress locks ecosystems in stagnation [60].

Research highlights the synergy between computational and synthetic biology, where engineering and computational tools overcome traditional limits in studying biological systems [18,24,67-85]. One paper showed that aligner tools such as BLAST and SHiMP2 perform best for highly mutated viral sequences [84]. Another discovered that combining Archive and OMEX formats would help solve critical reproducibility challenges in computational biology [68]. Another paper found that populations that can produce and share extended phenotypes have a higher fitness than populations that can only produce them [74]. One study found that testing Quasispecies theory in vivo faces hurdles such as mutation rate limits and ethical barriers, but synthetic approaches offer workarounds [85]. Another study revealed that the evolution of dynamics demonstrates how adaptive strategies arise: synchronisation behaviours develop through frequency tuning [77]. Two studies discovered that the survival strategies hinge on the mutation rates and population size [79,80]. Two additional studies showed that adaptable memory architectures improve flexibility when guided by epistasis and functional constraints [67,70]. In another study, learning strategies (imitation vs. experience) interact with selection thresholds to shape traits like parental care [71]. Genetic entanglement, such as secretion-metabolism linkages, counters cheaters at high costs [75], whereas operon structures limit cheater proliferation [88]. Synthetic biology employs tools like FPGA models for self-repair [83] and microfluidics for phage assembly [82]. Computational frameworks, including Boolean networks, unravel plant signalling crosstalk [76], while multiscale models dissect ion channel dynamics [72]. Insights into protein complexity highlight trade-offs from gene duplication [18], and the Extended Phenotype Hypothesis refines fitness dynamics in social systems.

Studies [50-57,86] also show that DOs transform biomedical and environmental research by simulating different evolutionary and ecological processes. They reveal antibiotic resistance traps, guiding strategies like antibiotic cycling [52] and show how gradual environmental decline causes irreversible ecosystem collapse versus a faster recovery after sudden ecosystem collapse [55]. Researchers were also able to find links between ultrafine particles and Alzheimer's disease, which is characterised by amyloid beta aggregation [53]. Researchers were also able to show how DOs helped to improve the food safety [86], while metabolic modelling helped to predict *Mycobacterium tuberculosis*'s antibiotic stress responses [54]. Innovations like KNN-BSBL algorithms boost tumour imaging [57], and tissue studies clarify wound healing [51]. Environmentally, bio-inspired insulation slashes building energy use by 67.1% [56], and wastewater microbes enhance nutrient recovery via PHA storage [50].

Theme 5: How were findings using DOs relevant to biology?

DOs are a reliable tool for better understanding biology. Studies have used DOs to help understand key areas such as Evolutionary dynamics and adaptation, Genetic and genomic architecture, ecological and population dynamics, and biomedical applications. DOs help to understand Evolutionary dynamics and adaptation. A volume of studies [13,15-18,20-23,26,28,32-34,37,40-48,53,55,59,68,71,73,74,77,78,80,81,83,84] shows how DOs are relevant to biology by helping to connect theory and empiricism in evolutionary biology. This is done by helping to test universal principles, such as mutations and genetic trade-offs. It also gives researchers insights into the adaptation mechanism, allowing them to have a more concrete understanding of how specific adaptations work and how they appear. Secondly, they also play a role in understanding the genetic and genomic architecture [1,12,19,24,25,27,29,30,33,35,36,38,39,49,67,69,70,75,82,85]. DOs helped to bridge the gap between theoretical genetics and empirical biology by helping to test different principles of genome architecture, helping to model the evolutionary dynamics of mutation and lastly, the ability to control things such as the environment makes DOs an indispensable tool to prob into genetic and genomic evolution.

Thirdly, DOs also play a part in the ecological and population dynamics in biology [9,10,31,50,56,60-66,72]. This is because they can simulate different environments, providing a powerful platform for researchers to study things such as universal principles, explore "What if" scenarios, such as mass extinction or things such as novel mutations and lastly, they provide scalability for complex systems that would be impossible or too costly to do in real life. Lastly, DOs help researchers in biomedical applications [8,11,14,51,52,54,57,58,76,86]. They accelerate drug discovery and resistance management. Researchers can also test out personalised treatments through genomic simulations without harming the patient.

Discussion

DOs have shown to be highly beneficial in biological research and various other fields, as they play a key role in research as both a supplement to wet laboratory and an alternative to web laboratory experiments that are not typically possible [3]. More importantly. DOs allow researchers to test things that would usually raise ethical concerns, such as studies on antibiotic resistance [52], genome mutations [25], and extinction events, [42] to name a few. These are just a few examples of how DOs can help study areas that might not be traditionally possible due to ethical and financial concerns.

DOs also help to minimise the time and money spent testing only one hypothesis. Consequently, researchers can subsequently conduct wet lab experiments based on the findings from the DOs. This gives the researchers the flexibility to adjust the scale of the experiment [2,6]. They can use DOs to replicate and mutate things much faster than biological organisms, allowing them to run experiments that would require thousands of generations to complete in hours using a computer.

However, DOs do not replace wet labs; they work hand in hand to play a crucial role in helping to test various hypotheses without needing to worry about issues such as ethics, funding, and time constraints [6]. This is because they offer a fast, scalable, controlled, and efficient platform for researchers to investigate hypotheses. This enables researchers from various fields to pose deeper questions, design superior experiments, and gain a more profound understanding of topics such as evolution, biomedical and environmental applications, biomechanics, and movement modelling.

Conclusion

In this study, we systematically reviewed 80 studies pertaining to digital organisms by focusing on five questions; namely, (a) What was the study about? (b) What tool(s) was/were used? (c) How DOs helped in the study? (d) What was/were discovered from the studies? (e) How were findings using DOs relevant to biology? Our findings show a wide range of applications of DOs in seven main areas; namely, (i) biomechanics and movement modelling, (ii) evolutionary and adaptation, (iii) molecular and cellular evolution, (iv) ecology and community dynamics, (v) computational and synthetic systems, (vi) biomedical and environmental applications, (vii) theoretical and cross-disciplinary studies; with 39 of the 80 studies uses Avida as experimental platform.

Supplementary Materials

Supplementary materials can be downloaded at https://bit.ly/Digital_Organisms_SR.

Conflict of Interest

The authors declare no conflict of interest.

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